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A Manual for Scallop Culture in British Columbia

N. Bourne, C. A. Hodgson and J. N. C. Whyte

Department of Fisheries and Oceans
Biological Sciences Branch
Pacific Biological Station
Nanaimo, British Columbia V9R 5K6

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A MANUAL FOR SCALLOP CULTURE IN BRITISH COLUMBIA

by

N. Bourne, C. A. Hodgson[1] and J. N. C. Whyte

Department of Fisheries and Oceans

Biological Sciences Branch

Pacific Biological Station

Nanaimo, British Columbia V9R 5K6

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[1] British Columbia Ministry of Agriculture and Fisheries
Aquaculture and Commercial Fisheries Branch
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ABSTRACT

Bourne, N., C. A. Hodgson and J. N. C. Whyte. 1989. A manual for scallop culture in British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1694: 215 p.

Scallops are highly prized seafood. In 1986 world landings were about 500,000 t (tonnes) and over half this production was from culture or enhancement operations. Scallop resources in British Columbia are too small to support a large sustained fishery and development of a sizable industry will have to rely on culture operations. In 1981, a program was initiated to investigate the feasibility of scallop culture in British Columbia with major focus on production of large numbers of juveniles in hatcheries. Work was primarily on two species, the exotic Japanese scallop, *Patinopecten yessoensis* and the native rock scallop, *Crassadoma gigantea*.

Scallop culture has received considerable interest in British Columbia, partly because of results of this program. This publication describes results of some of the work in the program and is intended as a manual for scallop culture for the industry. Details describing the biology of scallops, design and location of hatcheries, operations of hatcheries and nurseries and initial stages of growout work are provided.

Key words: Scallop, *Patinopecten yessoensis*, *Crassadoma gigantea*, culture, hatchery, nursery, growout

RÉSUMÉ

Bourne, N., C. A. Hodgson and J. N. C. Whyte. 1989. A manual for scallop culture in British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1694: 215 p.

Les pétoncles sont un fruit de mer très recherché. En 1986, les débarquements mondiaux ont atteint environ 500,000 tonnes métriques, dont plus de la moitié provient des opérations de culture ou de mise en valeur. Les ressources en pétoncles de la Colombie-Britannique sont trop réduites pour permettre une pêche soutenue de grande envergure; c'est pourquoi, la création d'une industrie d'une bonne taille doit passer par les opérations de culture. En 1981, un programme a été mis sur pied pour étudier la faisabilité de la culture des pétoncles en Colombie-Britannique en mettant l'accent principal sur la production en grands nombres de juvéniles dans des écloseries. Les travaux ont porté principalement sur deux espèces, le pétoncle japonais, Patinopecten yessoensis, qui est une espèce exotique, et le pétoncle de roches, Crassadoma gigantea, qui est une espèce indigène.

La culture des pétoncles a suscité considérablement d'intérêt en Colombie-Britannique, en partie à cause des résultats de ce programme. La présente publication décrit les résultats d'une partie du travail réalisé dans le cadre de ce programme et elle se veut un manuel sur la culture des pétoncles destiné à l'industrie. On y trouve des détails sur la biologie des pétoncles, la conception et le choix de l'emplacement des écloseries, sur les opérations des écloseries et des nurseries et sur les premiers stades de l'affinage.

Mots-clés: pétoncle, Patinopecten yessoensis, Crassadoma gigantea, culture, écloserie, nurserie, affinage

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1. INTRODUCTION

World scallop resources support large and valuable industries; in 1986, landings totalled 509,955 t (tonnes) whole weight (Anon 1988). One species, the Japanese scallop, Patinopecten yessoensis, accounted for 49% of the landings, and the Atlantic sea scallop, Placopecten magellanicus, accounted for another 25.5%. Over 50% of the 1986 world production resulted from culture or enhancement and a considerable literature has accumulated on the subject (Hodgson et al 1988).

Thirteen species of scallops occur in British Columbia waters but most are small or rare (Table 1) (Bernard 1983, Bourne 1988). Four species are either large enough or occur in sufficient abundance to elicit enquiries about the establishment of commercial fisheries. The four species are:- weathervane, Patinopecten caurinus; rock, Crassadoma gigantea; pink, Chlamys rubida; and spiny, Chlamys hastata (Figure 1).

Weathervane scallops are large, up to 250 mm shell height. A small commercial fishery exists for this species in Alaska and Oregon (Bourne in press). There are two small populations in British Columbia, one in the Gulf Islands area of the southern Strait of Georgia and the other in Dixon Entrance off the north coast of the Queen Charlotte Islands (Figure 2). Small local populations have been reported from a few other locations along the coast. British Columbia populations of weathervane scallops are too small to support a sustained fishery.

Rock scallops become large and massive and are usually found firmly cemented to rock surfaces. Their distribution is sporadic with abundance low in any one locality, although they are generally more abundant on the outer coast than in the Strait of Georgia. They do not lend themselves to a dragging-type fishery but attempts have been made to harvest them by diving. At present in British Columbia, they can be harvested only in the recreational fishery and catch is regulated by a daily catch limit.

Pink and spiny scallops are small, rarely larger than 80 mm shell height, and occur in small beds scattered throughout British Columbia waters. A small fishery began for both species in 1981, with the highest landing of 66 t occurring in 1987 (Table 2) . Fishing is by diving or towing small drags. It is unlikely that annual landings of these species will exceed 500 t.

Scallop resources in British Columbia are insufficient to support a large sustainable commercial fishery. The development of a significant scallop industry in the Province (annual landings of 5,000 t or more) must rely on culture technology. Considerable interest exists in scallop culture in British Columbia because of strong markets and the success of scallop culture in Japan (Taguchi 1978, Ventilla 1982).

In 1981, a program was initiated at the Pacific Biological Station to investigate the feasibility of scallop culture in British Columbia. This program is jointly sponsored by the Federal Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station and the British

Table 1. Species of scallops reported from British Columbia coastal waters (after Bernard 1983).

Chlamys hastata	spiny scallop
Chlamys rubida	pink scallop
Crassadoma gigantea	rock scallop
Patinopecten caurinus	weathervane scallop
Chlamys albida	
Chlamys behringiana	
Chlamys jordani	
Cyclopecten argenteus	
Cyclopecten carlottensis	
Cyclopecten squamiformis	
Cyclopecten knudseni	
Delectopecten randolphi	
Delectopecten vancouverensis	

Table 2. Landings in tonnes (whole weight) and value in dollars of commercial scallop landings in British Columbia, 1982-1987. Landings are spiny and pink scallops except where noted.

Year	Landed Weight	Landed Value
1982	8.0	-
1983	11.0	45,000
1984	6.5*	74,000
1985	52.7*	137,000
1986	62.1	277,000
1987	66.1	250,000

* Includes 1.4 t and 0.3 t of weathervane scallops in 1984 and 1985 respectively.

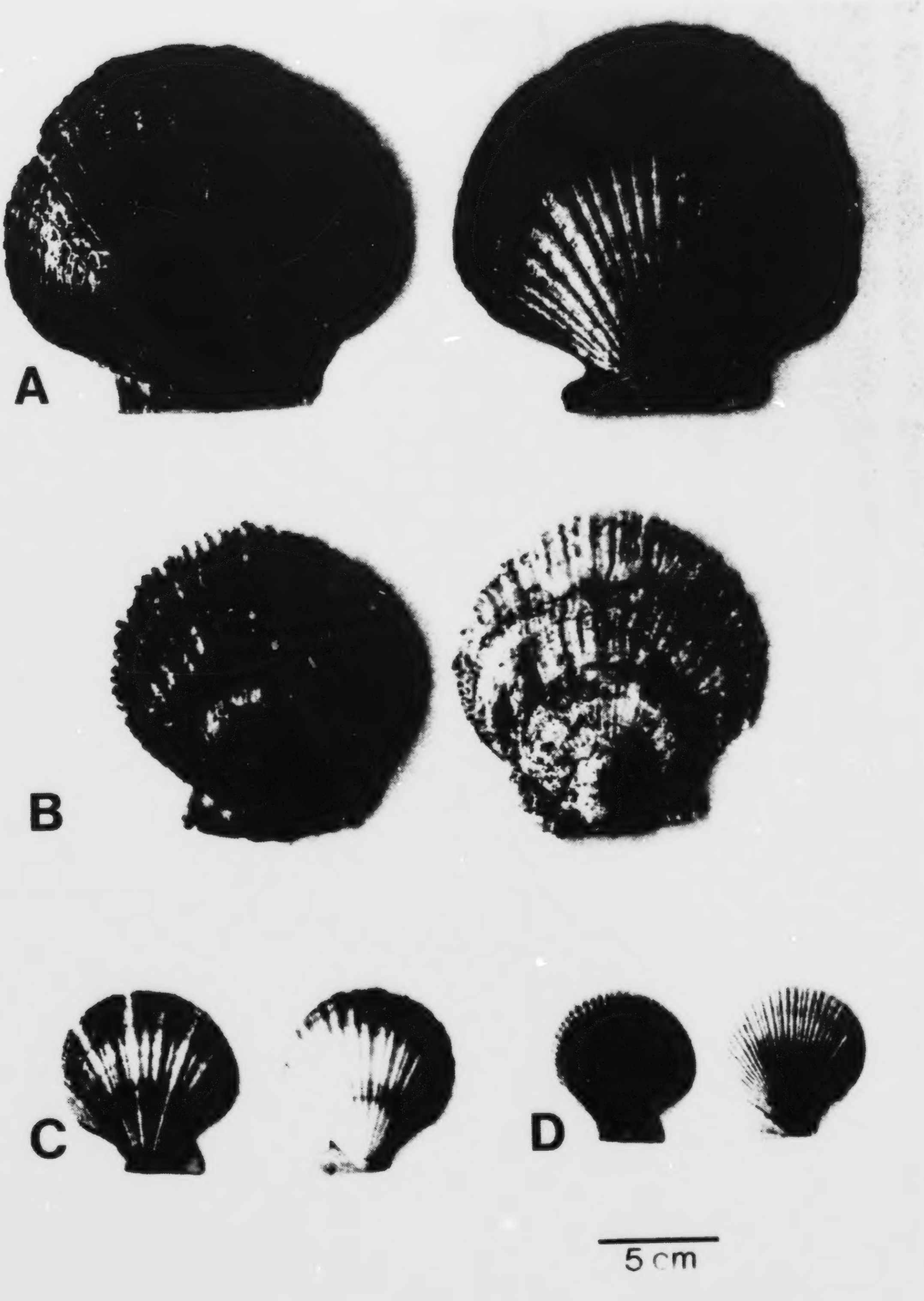

Figure 1. Four species of scallops that occur in British Columbia waters and have been harvested commercially. a) weathervane, Patinopecten caurinus, b) rock, Crassadoma gigantea, c) spiny, Chlamys hastata, and d) pink, Chlamys rubida. Valves on the left are the upper left valves, those on the right are the lower right valves.

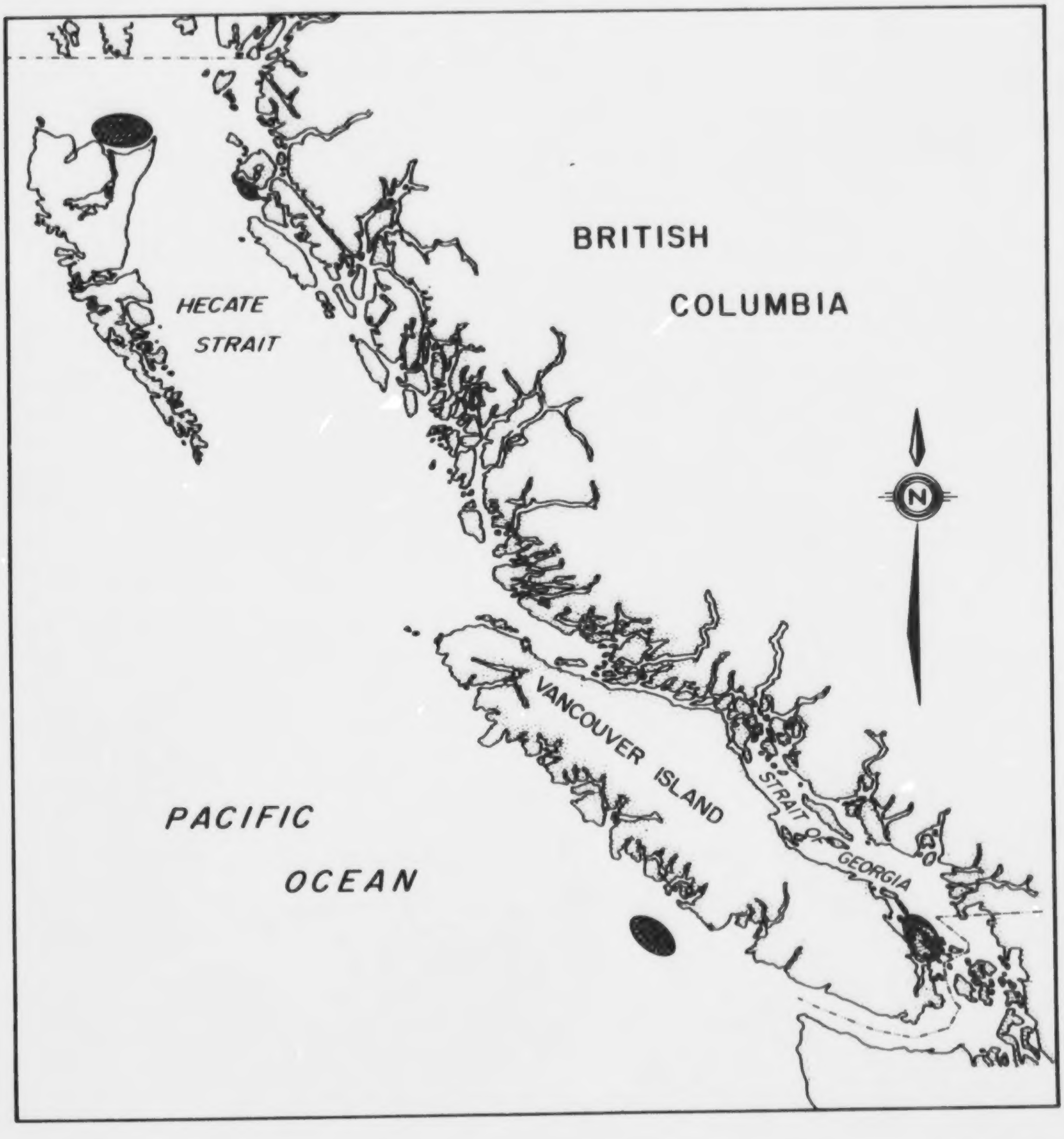
BRITISH
COLUMBIA
HECATE
STRAIT
N
VANCOUVER ISLAND
STRAIT OF GEORGIA
PACIFIC
OCEAN

Figure 2. Location of main weathervane scallop beds in British Columbia coastal waters.

Columbia Ministry of Agriculture and Fisheries (originally support was from the Ministry of Environment).

This publication summarizes much of the work done in this program, which has focused mainly on developing hatchery and nursery technology to produce quantities of juveniles (seed) for growout operations. The collection of natural sets of juvenile scallops and initial growout work also is described.

The publication was compiled as a working manual for scallop culture in British Columbia. References are provided so that additional information can be obtained. Each section is a complete unit so that the user can study a particular topic, e.g. holding larvae, without having to read the entire publication. This has led to some unavoidable repetition. Several scientific terms have been used in the manual. A glossary has been provided for a quick explanation of these terms.

2. BIOLOGY

Some knowledge of the biology of scallops is necessary to understand scallop culture technology and to assist in solving problems. The following is a general description of the biology of species used in experimental culture work at the Pacific Biological Station.

2.1 External Anatomy

Anatomical orientations used to describe fish and mammals are used also in describing scallops (Figure 3). The hinge area is the dorsal region of the animal and the region of the shell opposite the hinge is the ventral margin. The mouth is located anteriorly. There is a right and left valve. The four native species mentioned above lie naturally on the lower right valve. Valve height is the straight line distance measured vertically from the hinge to the ventral shell margin; valve length is the straight line distance from the anterior to the posterior shell margins. Valve shape is variable. The top left valve of weathervane and rock scallops is flat and the lower right valve is convex or cupped. Both valves of pink and spiny scallops are convex. The exterior surface varies with species; it can be smooth, have smooth ridges or have ribs that may be smooth or studded with spines.

The valves are joined by a ligament along the dorsal margins at the hinge. On the interior surface there is a pit in the centre of the hinge containing a dark elastic pad, the resilium. The resilium and the hinge spring the valves open when the adductor muscle relaxes.

There are two protrusions at the hinge called auricles (sometimes called ears or wings) that give the animal a long hinge line. On the lower right valve there is an indentation in the anterior auricle called the byssal notch. Auricles on the upper left valve may be of equal size as in weathervane scallops or unequal as in rock, pink and spiny scallops.

The interior surface is smooth and usually white, although there is a deep purple colour near the hinge in rock scallops. Near the centre of the valve the circular muscle attachment scar is visible. About one third of the way in from the edge of the valve there is a shallow indistinct groove, the pallial line, that marks the attachment of the mantle (Figure 3).

2.2 Internal Anatomy

If the upper valve is carefully removed, the soft inner parts are seen.

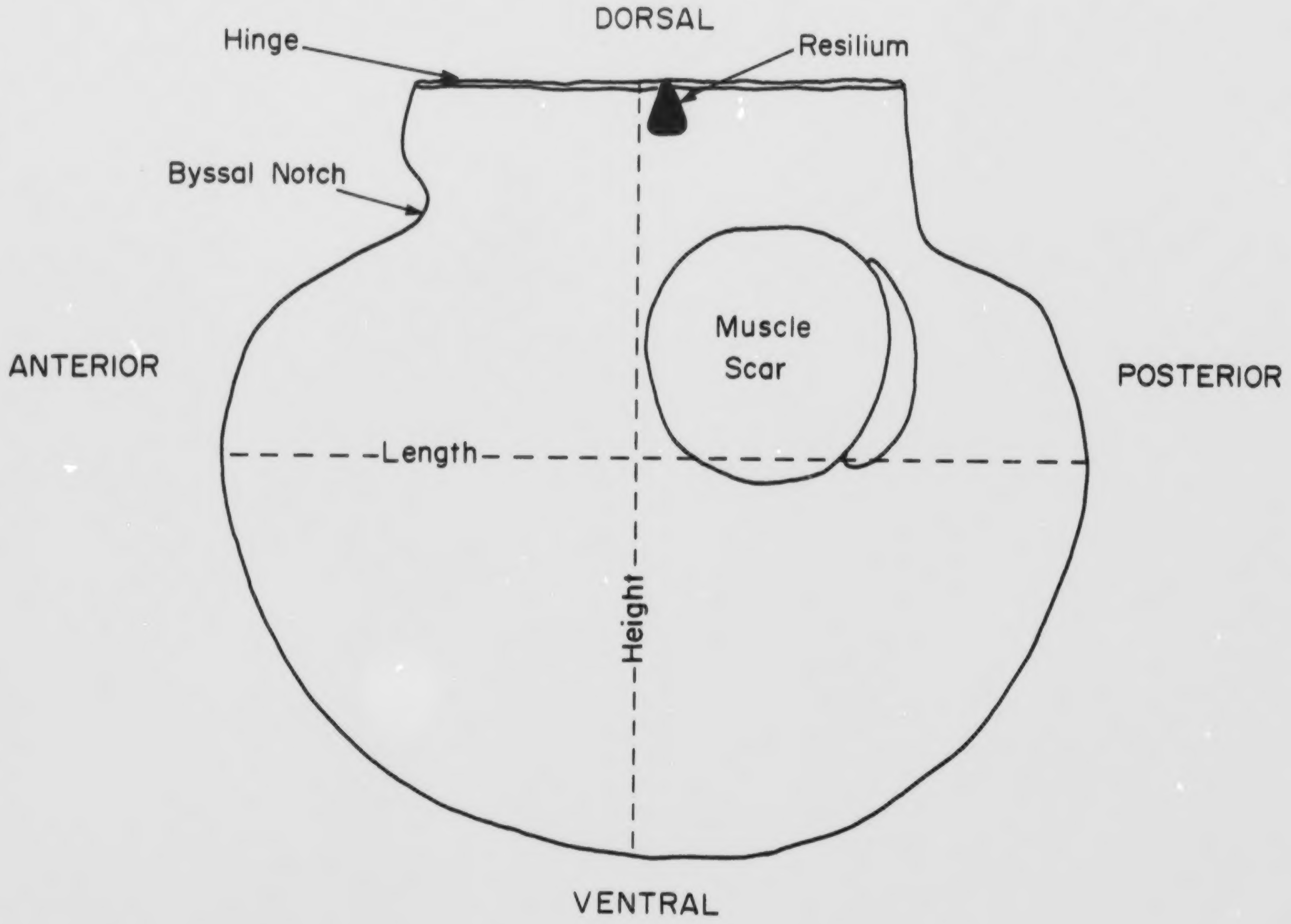
DORSAL
Hinge
Resilium
Byssal Notch
ANTERIOR
Muscle Scar
POSTERIOR
Length
Height
VENTRAL

Figure 3. Inner surface of right valve of a Japanese scallop showing parts of the shell and their relationship to anterior, posterior, dorsal and ventral regions.

2.2.1 Mantle

A thin, almost transparent sheet of tissue with a thickened margin envelopes the body. This is the left half of the mantle, the right half is against the right valve. Both mantle halves are attached to the shell from the hinge ventral to the pallial line but free at their edges.

The thickened edge of the mantle is pigmented and has three folds. Numerous eyes occur on the middle fold and function to detect shadows or movement. A row of tentacles, some of which are quite long, is also present on the middle fold. These are primarily feeling or tactile organs. The inner fold has a row of short tentacles which are mainly chemoreceptors. This fold forms the velar fold, sometimes called the "hanging curtain", which is the visible part of the mantle when the scallop is in the normal resting state.

The mantle has several functions; it secretes the shell, assists in respiration, has highly developed sense organs, controls the inflow and outflow of water, and controls movement when the animal swims.

2.2.2 Adductor Muscle

Removal of the left half of the mantle shows the centrally located large, white, circular adductor muscle (Figure 4). The large anterior portion is the "quick muscle" and contracts to snap the valves shut when the animal is disturbed or when it is swimming. The smaller posterior part known as the "catch muscle" holds the valves in position when they have been closed or partly closed by the quick muscle.

2.2.3 Gills

Circling part of the muscle is a pair of crescent-shaped gills. The gills are composed of four demibranchs, two on each side of the body. Along with the mantle, they are used for respiration and are important in feeding.

2.2.4 Urogenital System

The gonad is a conspicuous organ lying in a semi-circle around the ventral and anterior part of the adductor muscle. The four scallop species discussed here are dioecious, ie., the sexes are separate, although hermaphroditic individuals may occur (Hennick 1971; Jacobsen 1977). Unless the gonad is completely empty of sex cells, the sexes are easily distinguished; the female gonad is orange-red to red in colour and the male is creamy white.

Two kidneys lie flattened against the anterior part of the adductor muscle (Figure 4). They are small, thin, brown sac-like bodies. The kidneys empty through large slits into the mantle chamber. Eggs or sperm from the gonads are extruded through ducts into the lumen of the kidney and then into the mantle chamber.

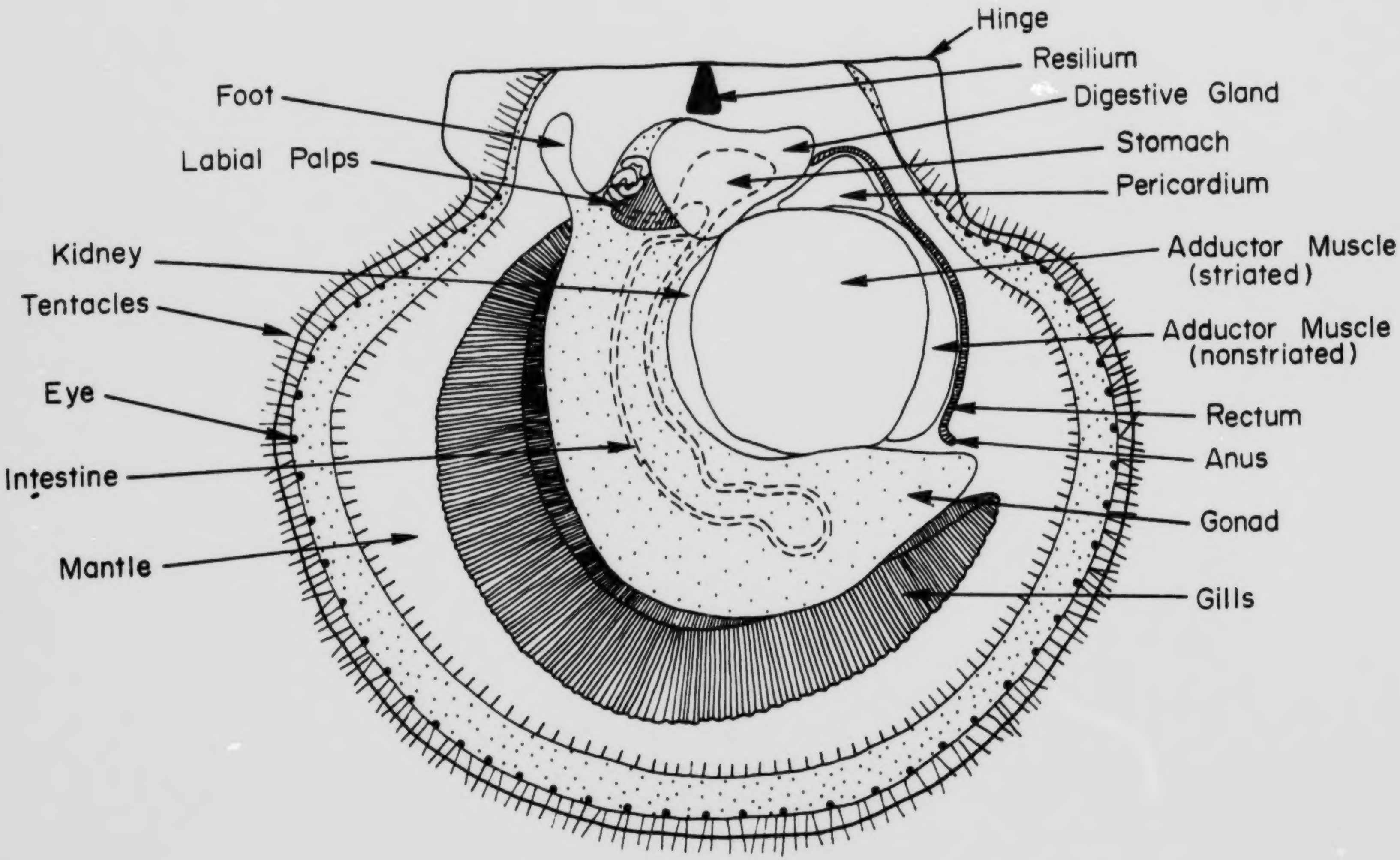
Hinge
Resilium
Digestive Gland
Stomach
Pericardium
Adductor Muscle (striated)
Adductor Muscle (nonstriated)
Rectum
Anus
Gonad
Gills
Foot
Labial Palps
Kidney
Tentacles
Eye
Intestine
Mantle

Figure 4. Japanese scallop with right valve, mantle lobe and gills removed to show internal anatomy.

2.2.5 Foot

At the anterior end of the gonad is the small, white-coloured foot. In larval and juvenile stages it is used for locomotion but in the adult it is rudimentary and has little function. Half way along the foot is the opening from the byssal gland through which the animal secretes a thread-like, elastic substance called "byssus" by which it can attach itself to a substrate. Some species, such as pink and spiny scallops, attach themselves to a substrate by byssus throughout their life but in others, such as weathervane scallops, the ability is lost in older animals.

2.2.6 Digestive System

Between the foot and hinge are the leaf-like labial palps (Figure 4). These surround the mouth and direct food collected by the gills into the mouth. A short oesophagus leads from the mouth to the stomach which is entirely surrounded by the digestive diverticulum, a dark mass of tissue between the adductor muscle and hinge. The stomach can be seen if the digestive diverticulum is gently teased away. The intestine leads out of the stomach, loops through the gonad, back around the adductor muscle to form the rectum, which goes through the pericardium and ventricle and hooks back to end in the anus.

Lying partly in the stomach and partly in the section of intestine which enters the gonad is the crystalline style. It is amber coloured, translucent, and rod-shaped, and is easily seen if the stomach or front part of the intestine is cut. The crystalline style churns food in the stomach and releases an enzyme which assists in digestion. If scallops are held out of water for a few hours the crystalline style becomes much reduced or disappears, but is reconstructed quickly when the animal is replaced in the water.

2.2.7 Circulatory System

Scallops have a simple circulatory system. The heart lies in a transparent sac, the pericardium, close to the adductor muscle (Figure 4). It consists of two irregular-shaped auricles and a ventricle which is transversed by the rectum. An anterior and posterior aorta lead from the ventricle and carry blood to all parts of the body. The venous system is a vague series of thin-walled sinuses through which blood returns to the heart.

2.2.8 Nervous System

The nervous system is difficult to observe without special preparation. Essentially it consists of three pairs of ganglia with connectives (cerebral, pedal and visceral ganglia).

2.3 Life History

The following is a general description of the life history of scallops and differences between species are noted where appropriate.

2.3.1 Gonadal Development and Spawning

As with most bivalves, sexual maturity is a matter of size rather than age (Quayle and Bourne 1972). Weathervane scallops are sexually mature at about 70 mm shell height, pink and spiny scallops at a shell height of 35 mm shell height (Bourne, In press). Rock scallops are sexually mature when they are 70 mm shell height (MacDonald and Bourne, In press).

The mechanisms that initiate the production of eggs and sperm (gametogenesis) and trigger spawning in the natural environment are poorly understood, but temperature is undoubtedly important. The period of natural spawning differs for species and geographic location. In the Gulf Islands area, weathervane scallops begin to develop gametes in late fall. By early April the gonads are full and gonaducts are obvious. Spawning occurs from late April to mid-June (MacDonald and Bourne 1987). Spawning is protracted and the animals are intermittent spawners, releasing eggs or sperm over an extended period of time. Gonads without gametes are not observed until July.

Off Oregon, the spawning season of weathervane scallops extends from January through June (Robinson and Breese 1984). In Alaska gonadal development occurs from October to May and a single spawning occurs from early June until mid-July (Hennick 1970). Rock scallops spawn over an extended period from October to January in southern California (Jacobsen 1977) and from June to August in Puget Sound (Lauren 1982). In British Columbia it is believed rock scallops spawn from June to October (MacDonald and Bourne, in press). Initial observations indicate that spiny scallops spawn from August to October and pink scallops from January to March in southern British Columbia (Bourne and Harbo 1987).

Both eggs and sperm are expelled through two ducts into the kidney where they pass into the lumen and are expelled into the mantle cavity through the openings in the kidney. Sperm is released in a steady stream through the exhalant opening of the mantle. Eggs are also emitted in this fashion and periodically females clap their valves and expel eggs from the ventral portion of the mantle cavity. This may be done to clear eggs lodged on the gills.

After spawning, gonads of weathervane scallops are clear and colourless and it is not possible to sex animals visually. The gonads remain in the resting stage until gametogenesis is again initiated in the late fall. In rock, pink and spiny scallops the gonads never appear to empty completely and it is possible to sex animals by gonad colour throughout the year.

2.3.2 Embryonic and Larval Development

Fertilization of eggs occurs in the water outside of the adults, after they have been released. At fertilization, the egg undergoes meiotic division to reduce the number of chromosomes to a haploid number before the male and female pronuclei can fuse to form the zygote (Figure 5). Two polar bodies are released during meiotic division. At least one polar body is visible and, once apparent, indicates that the egg is successfully fertilized. Eggs are heavier than water and sink to the bottom. Cell division begins and within 30 minutes the egg has divided into the two cell stage, called first division. As with all mollusc species, a polar lobe is formed during first division (Figure 5). Often, a zygote appears as though it has divided into three instead of two cells. This process is normal and eventually the lobe joins with one of the resulting cells, causing one cell to be slightly larger than the other.

The time of embryonic and larval development is temperature and species specific. Within 24 hours, the fertilized egg has passed through the multi-celled blastula and gastrula stages, and within 36 hours has developed into a motile trochophore. The trochophore is somewhat oval in shape, about 60-80 um (micrometre) in size, and has a row of cilia around the middle and a long apical flagellum. Within 72 hours, the embryo develops into a larva.

The early larval stage is sometimes referred to as the straight hinge, "D", or Prodissoconch I stage (Figures 6, 7, and 8). The larva has two valves, a complete digestive system and a velum, an organ peculiar to molluscan larvae. The velum is ciliated along its outer margin and enables the larva to swim, but only well enough to maintain itself in the water column. When the larva swims through the water column, the velum collects unicellular phytoplankton upon which the larva feeds.

Larvae continue to swim and feed in the water and develop into umboned or Prodissoconch II larvae (Figures 7 and 8). This stage lasts about 3 weeks at which time they are mature. Size at maturity is species specific.

When the larva nears maturity, a well developed foot, a pair of eyespots, and gill rudiments become evident. Between periods of swimming, the larva settles and crawls on a substrate by use of its foot. When a suitable substrate is found, the larva secretes a byssus from the byssal gland to serve as a temporary holdfast. The larva is now ready to metamorphose.

2.3.3 Metamorphosis

Metamorphosis is a critical time in the development of bivalve larvae and considerable mortalities can occur at this time. At metamorphosis larvae undergo major anatomical changes. The animal changes from a pelagic to a benthic existence (Figure 9). Larval organs such as the velum and anterior adductor muscle are lost. Further, the soft parts of the body undergo an anterior rotation. This results in the foot shifting from a posterior to an anterior position (Hodgson and Burke 1988). During metamorphosis, the animals live on food reserves accumulated during the larval period. The gills develop and serve as feeding organs about two weeks after metamorphosis. The animal is now a juvenile, or spat, and continues to grow and develop until it reaches the adult stage (Figure 10).

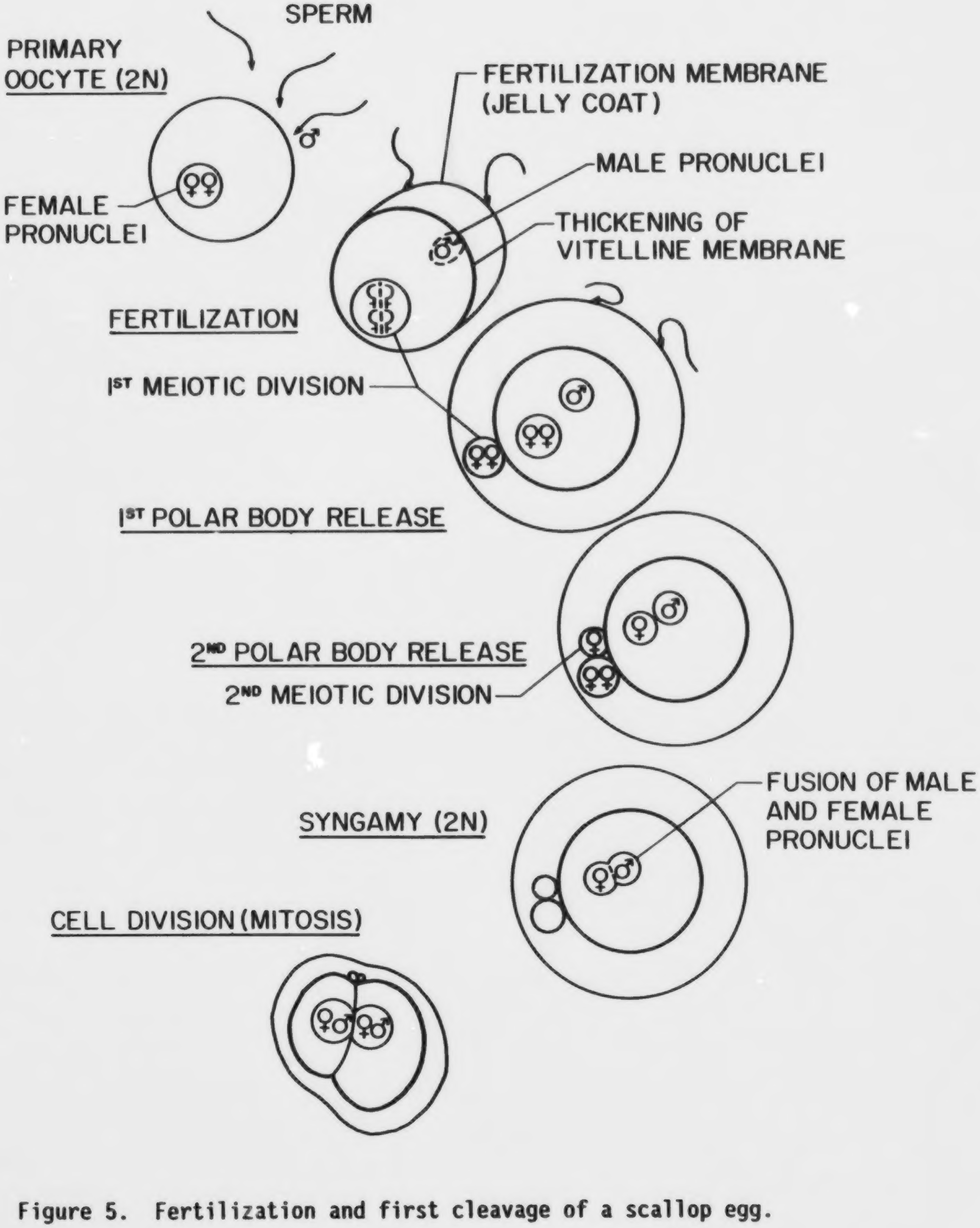
SPERM
PRIMARY OOCYTE (2N)
♂
FEMALE PRONUCLEI
♀♀
FERTILIZATION MEMBRANE (JELLY COAT)
MALE PRONUCLEI
THICKENING OF VITELLINE MEMBRANE
FERTILIZATION
1ST MEIOTIC DIVISION
1ST POLAR BODY RELEASE
2ND POLAR BODY RELEASE
2ND MEIOTIC DIVISION
SYNGAMY (2N)
FUSION OF MALE AND FEMALE PRONUCLEI
CELL DIVISION (MITOSIS)

Figure 5. Fertilization and first cleavage of a scallop egg.

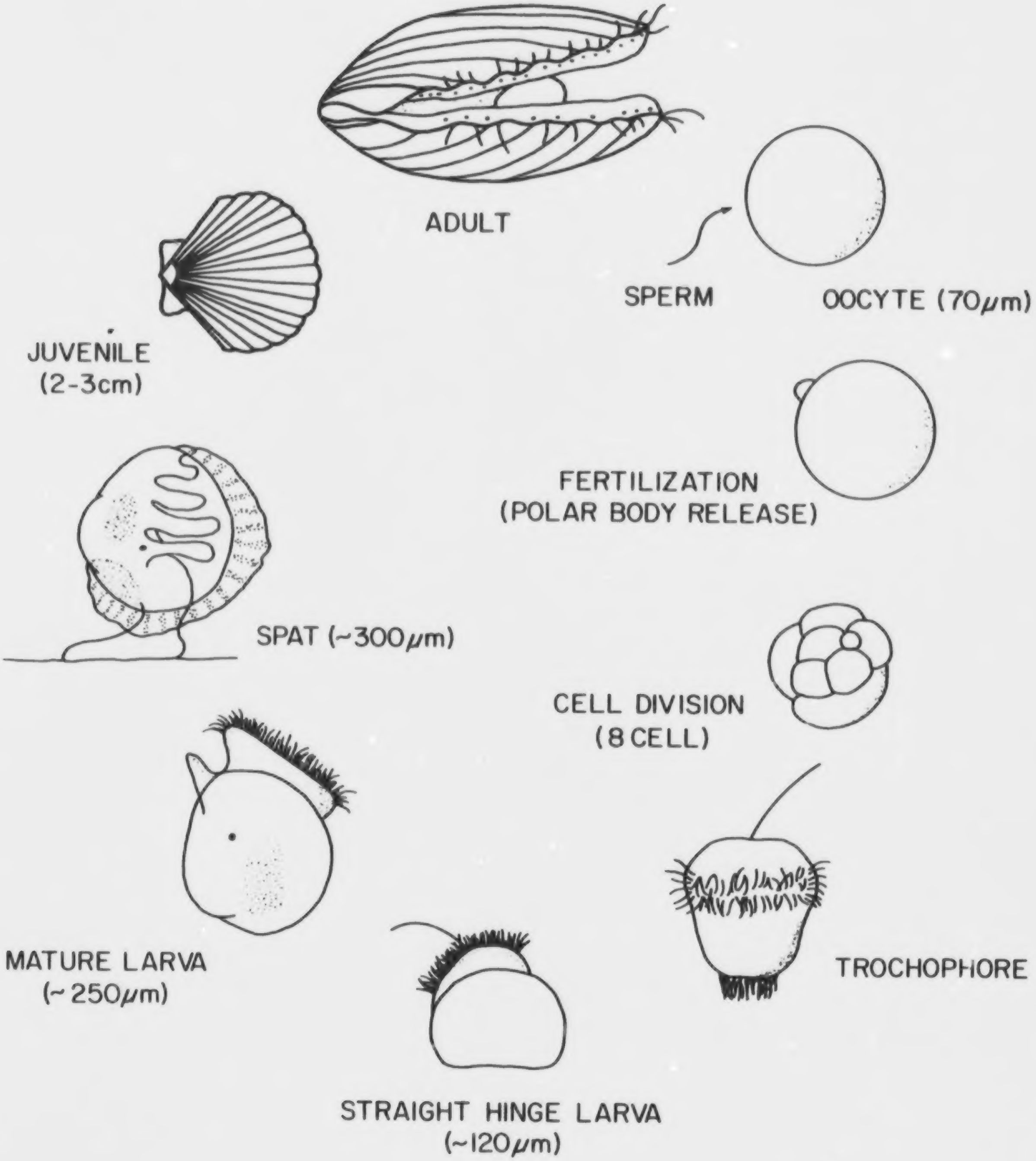
ADULT
SPERM
OOCYTE (70μm)
JUVENILE
(2-3cm)
FERTILIZATION
(POLAR BODY RELEASE)
SPAT (~300μm)
CELL DIVISION
(8 CELL)
MATURE LARVA
(~250μm)
TROCHOPHORE
STRAIGHT HINGE LARVA
(~120μm)

Figure 6. Life history of a scallop.

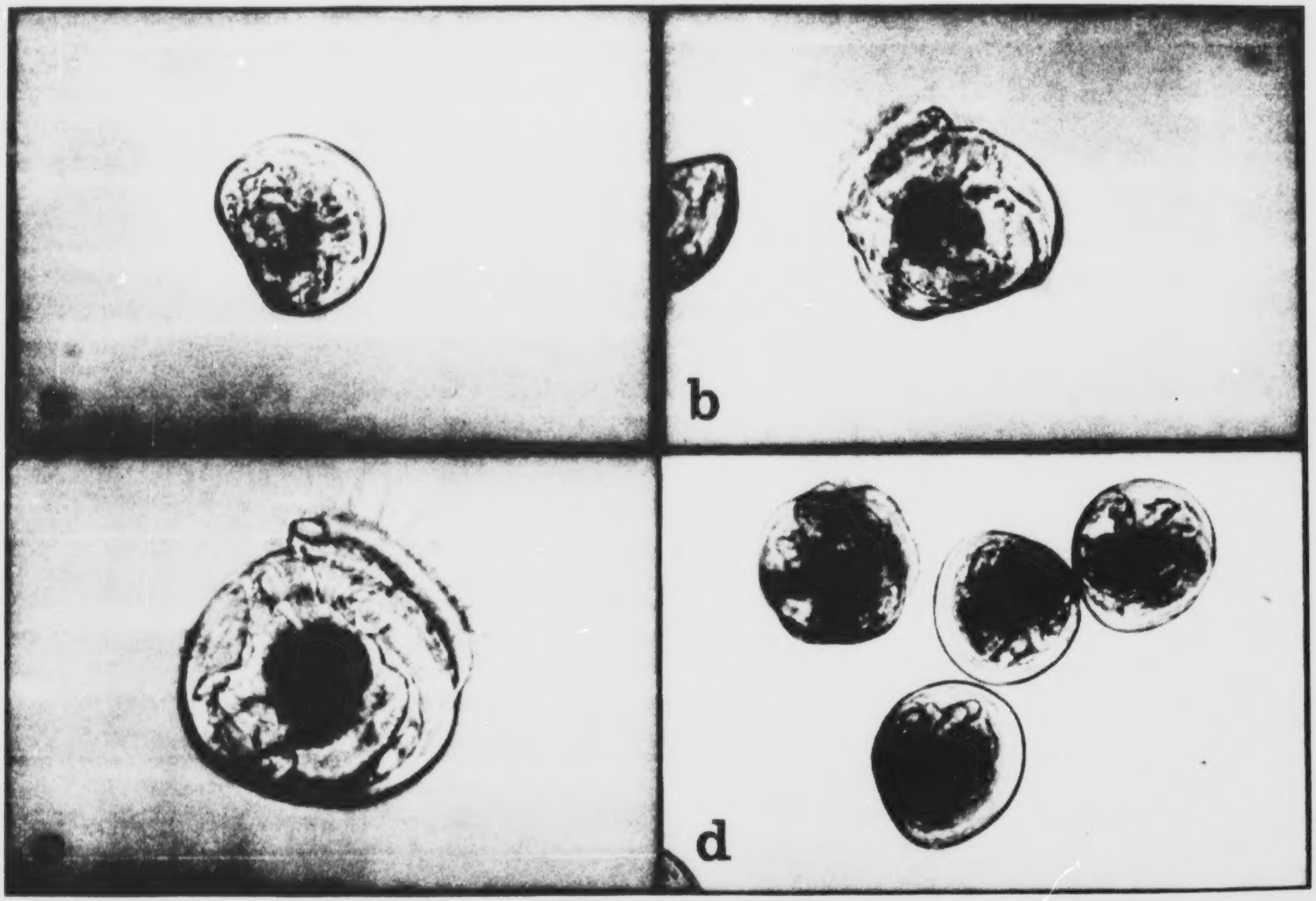

Figure 7. Japanese scallop larvae at different stages of development. a) 5 days old, 119 um shell length b) 8 days, 144 um c) 20 days, 225 um d) mature larva, 22 days, 265 um.

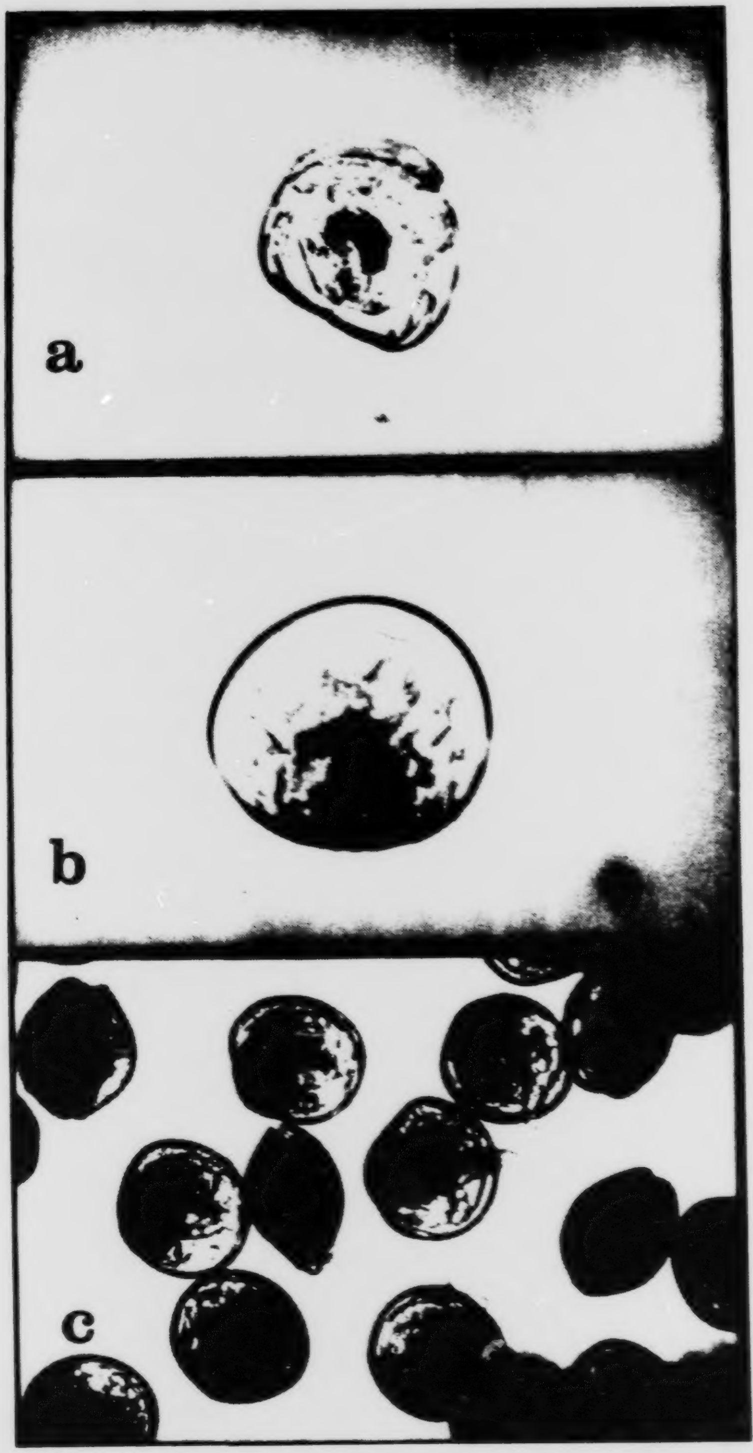

Figure 8. Rock scallop larvae at different stages of development. a) 6 days, 121 um shell length b) 14 days, 153 um c) mature larva, 19 days, 215 um.

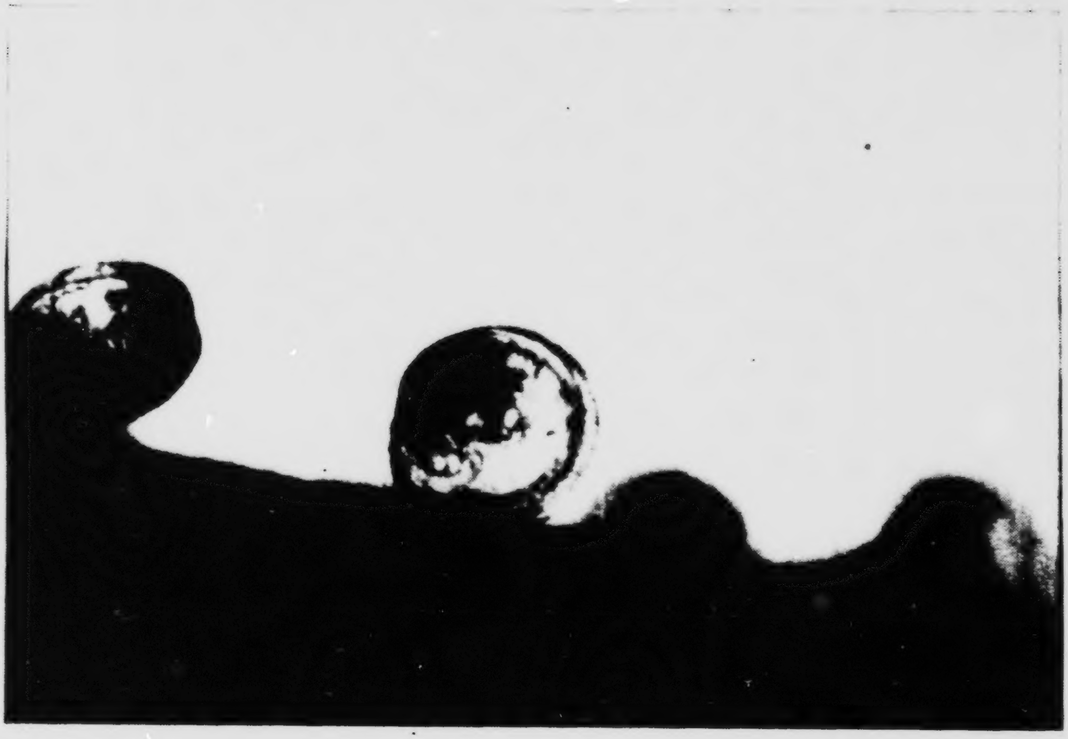

Figure 9. Rock scallop post-larva or spat, 3 days after metamorphosis. 223 um shell length.

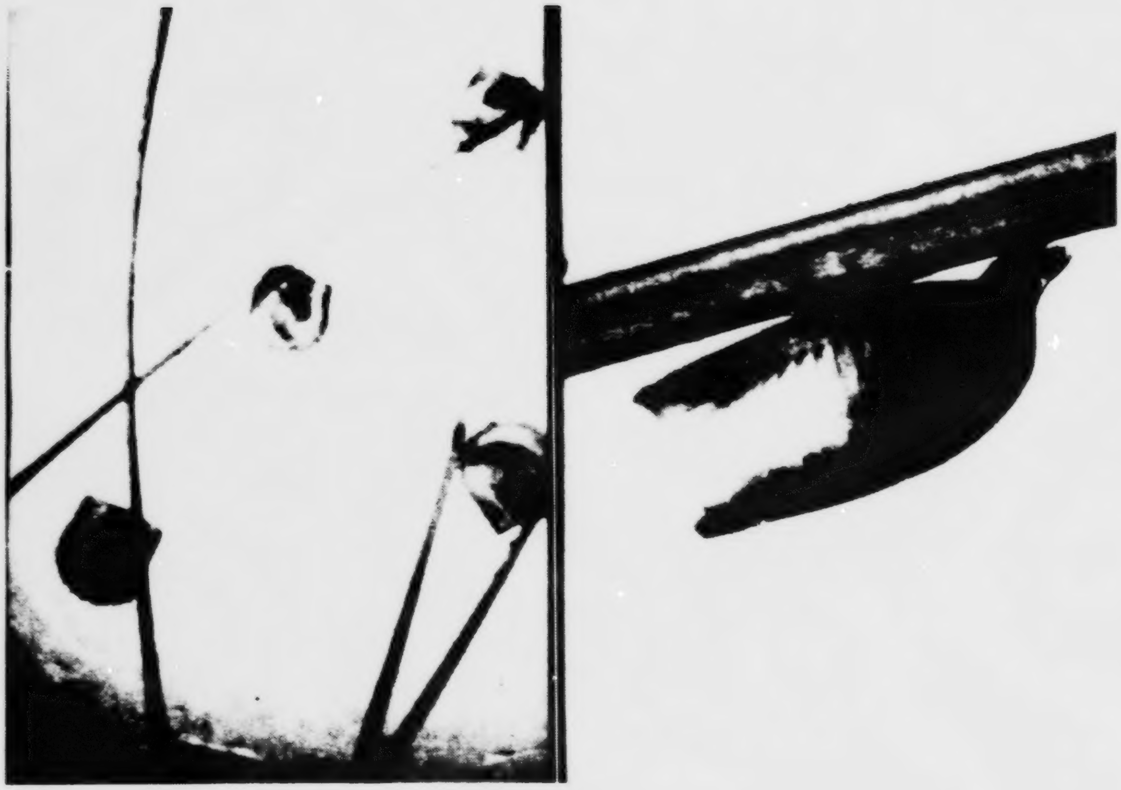

Figure 10. Japanese scallop spat, 4-5 mm shell height.

2.4 Feeding

Scallops, like most other bivalves, are filter feeders. The ctenidia or gills are well developed and serve as feeding and respiratory organs. The ctenidia are covered with cilia, tiny vibrating hairlike structures whose concerted beat produces a water current. When resting normally on the bottom there are two openings between the edges of the mantle, one at the anterior end, which is the inhalant opening, and one at the posterior end, the exhalant opening. Water is drawn through the inhalant opening into the mantle chamber, through the gills, and out the exhalant opening. The gills collect any plankton (minute plants and animals, 2-70 um in size) and bind it in mucous. Strands of food-laden mucous are passed vertically and then anteriorly by means of ciliary action along special grooves on the gills to the labial palps, which assist in directing the food masses into the mouth. Scallops can exercise some selection of their food and periodically the palps reject small masses of food, called "pseudo-faeces". Pseudo-faeces periodically are expelled from the mantle cavity by violent valve clapping.

The best types of food are not known. Also, the value of fine particles of non-living organic material (detritus) and associated bacteria may be important as food.

2.5 Growth

Growth of bivalves is measured in several ways, including increase in shell size, increase in weight of soft body parts, or a combination of the two. Generally, increases in shell height or length are used as a measure of growth. Growth generally is rapid during spring and summer when abundant food is present and water temperatures are warmer but decreases and virtually ceases in winter, forming annual checks (Figure 11). However, these winter growth checks must not be confused with other checks laid down during other times of the year, for example, checks caused by spawning or periods of stress. Most species of scallops can be aged by counting annual growth checks and growth rates can be estimated by measuring the distance between annual rings. Rock scallops cannot be aged in this manner since the rings are obliterated as the shell becomes massive.

Another method of aging scallops is to count annual rings that are laid down on the resilium (MacDonald and Bourne, in press). Although individual scallops can be aged by this method, growth rates cannot be calculated.

Growth varies with geographic area. In southern British Columbia, growth of weathervane scallops is faster in inshore areas than in offshore areas (MacDonald and Bourne 1987). It requires about three years for weathervane scallops to attain a shell height of 100 mm in inshore waters and about five years in offshore waters. Growth of weathervane scallops off Oregon was similar to that in the offshore area of British Columbia but growth

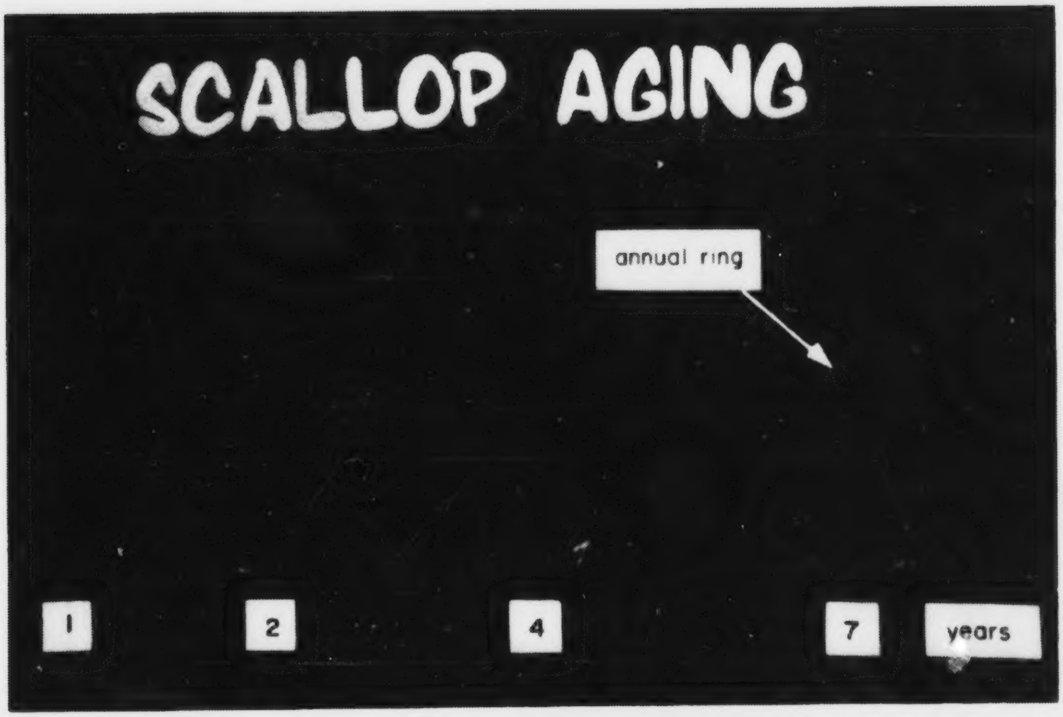
SCALLOP AGING
annual ring
1
2
4
7
years

Figure 11. Weathervane scallop shells showing location of annual winter growth checks.

in Alaska is similar to that in the inshore area of British Columbia. Weathervane scallops are long lived and individuals of 28 years have been reported (Hennick 1973).

Rock scallops grown in suspended culture in California attained a shell height of 120 mm in two years (Leighton 1979). In grow-out trials at the northern end of the Strait of Georgia in British Columbia, the largest individual grown in suspended culture measured 90-100 mm in three years, mean 80 mm (MacDonald and Bourne, in press). Rock scallops of 27 years of age have been recorded in British Columbia.

Pink and spiny scallops are slow growing. Spiny scallops attain a shell height of 75 mm in five years, pink scallops a shell height of 60 mm in about six years (Bourne and Harbo 1987). Pink and spiny scallops rarely live past six years.

2.6 Behaviour

Under normal conditions species of scallops described here lie on the bottom on their right valve; pink and spiny scallops are often attached to the substrate by byssus. The two valves are separated 10-15 mm at the ventral margins with the outer folds of the mantle meeting between the two valves. The tentacles are extended and the eyes can be seen easily. If the scallop is disturbed the mantle and tentacles are quickly withdrawn and the valves are snapped shut. When disturbed, the animal frequently spins in its place and faces in a different direction.

One interesting feature unique to scallops is their ability to swim. Even juvenile rock scallops, up to 30 mm shell height, can swim. Swimming results from a jet action. The animal opens its valves and takes in water. The valves and the mantle then are closed, and water is forced out through the inhalant and exhalant openings. The scallop is propelled forward (ventral margin first) and the process is repeated. Frequently, particularly if the scallop is disturbed or when taking off initially, it moves backward (hinge first) by forcing water out through the mantle at the ventral edge. Swimming is probably used mainly to avoid predators. Distances covered during individual swims are short, up to 10 meters.

2.7 Predators

Little is known about predators of scallops. Plankton feeders probably consume large quantities of larvae. Small weathervane scallops, 30-40 mm shell height, have been found in the stomachs of flounders from the Gulf Islands area in southern British Columbia. Crabs may be serious scallop predators in some situations. Juvenile and adult scallops can be preyed upon by several species of crabs, including the graceful crab, _Cancer gracilis_, Dungeness crabs, _C. magister_, and red rock crabs, _C. productus_. Seastars, particularly the sun star, Pycnopodia helianthoides and the mottled star, Evasterias troschellii, are abundant in subtidal areas and are undoubtedly important predators of scallops.

Shell boring worms, Polydora, burrow in the shell and weaken it and this may cause mortalities (MacDonald and Bourne 1987). The boring sponge, Cliona sp., may also cause mortalities.

It is not known if epidemic diseases cause mass mortalities but they may be important at times.

3. CULTURE

Considerable interest in scallop culture exists in British Columbia because of strong markets and the success of scallop culture in other parts of the world. Scallop resources in British Columbia are too small to support a large sustainable commercial fishery. If a significant scallop industry is to develop in British Columbia (annual landings of 10,000 t or more), it will have to rely on culture operations.

In any culture operation, a primary requisite is an abundant, reliable and inexpensive supply of seed (juveniles). In bivalve culture, seed can be obtained from two sources; collection of natural sets or from hatcheries.

3.1 Natural Sets

In most bivalve culture operations throughout the world seed is obtained from natural sets (Quayle 1988b). Seed can be gathered from areas of abundance or collectors can be placed out to gather metamorphosing larvae as they settle from the plankton.

Collecting mature or settling larvae from the plankton is used to obtain juvenile scallops for commercial and experimental culture operations in Japan, New Zealand, Australia and the U.K. (Ventilla 1982, Bull 1986, Cropp 1987, Paul 1987). Areas used to collect seed are monitored for oceanographic parameters such as water temperature, salinity and current. Gonadal development of adult scallops is followed to determine time of spawning. When spawning occurs, larval development is monitored by plankton sampling. Approximately two weeks before the larvae metamorphose, collectors are set out. The standard type of collector is the "Japanese collector", a mesh bag about the size of an onion bag, 80 by 40 cm, with a mesh size of about 3.0 mm. Collecting material (cultch), consisting of old gill net, monofilament, or other fibrous material is placed inside the bag. At settlement, scallop larvae swim through the mesh of the collectors and settle on the cultch. They remain attached to the cultch by byssus until they are about 10 mm shell height, at which time they drop off and are retained in the bags. The bags are harvested, the juvenile scallops removed and placed into intermediate culture.

The advantage of natural sets is cost. It is cheaper to collect juveniles from natural sets than to raise them in hatcheries. However, collecting natural sets has disadvantages. Recruitment may be erratic or so low that consistent and successful annual sets may not be obtained.

Attempts have been made to collect natural sets of juvenile scallops in British Columbia.

3.1.1 Weathervane Scallops

Three attempts were made to collect juvenile weathervane scallops from 1981-1983 in Trincomali Channel in the Gulf Islands area of southern British Columbia. In the first year, four lines with a total of 125 collectors were suspended 1-2 m off the bottom on horizontal longlines. In the second year, three horizontal bottom lines and two vertical lines with collectors at 2.5 m intervals were suspended. In the third year, one horizontal line and two vertical lines with collectors at 1.0 m intervals were put out at two localities in Trincomali Channel. In the first year (1981), ten juvenile weathervane scallops were collected. In the second year, one juvenile was collected, and in the final year no animals were collected. In 1982 and 1983 there were heavy sets of the crab, Cancer oregonensis, up to 50 per collector. These would have eaten any juvenile bivalves that settled in the bags.

Experience indicates it is unlikely there are any areas in British Columbia where sufficient quantities of juvenile weathervane scallops can be collected to support culture operations.

3.1.2 Rock Scallops

Some success has been achieved collecting juvenile rock scallops from natural sets. They are frequently caught on oyster strings in British Columbia when hanging culture methods are used. In scallop seed collecting operations in Barkley Sound, reasonable numbers of juvenile rock scallops were found in collectors along with juvenile pink and spiny scallops. It was estimated that 25% of the scallops collected were rock scallops (G. Lindsey, Pers. Comm.). Further research is required to determine if there are natural breeding areas of rock scallops in British Columbia that could be used for a consistent seed supply for a large commercial rock scallop culture operation. It may be too costly to separate rock scallop juveniles from the other scallop species.

3.1.3 Pink and Spiny Scallops

Pink and spiny scallops can be collected in quantity from natural sets, up to 2,000 juveniles per collector (H. Miller, Pers. Comm.). However, these species probably are too small and too slow growing to support economical culture operations.

3.2 Production of Juveniles in Hatcheries

The alternative to obtaining a supply of juvenile bivalves from natural sets is to produce them in hatcheries. The lack of consistent natural sets of scallops in British Columbia indicated a requirement to investigate the feasibility of developing a hatchery system.

Commercial hatcheries that produce several species of bivalves are well established and can be of various size, depending on intended levels of production. A bivalve hatchery includes facilities to condition and spawn adults, and to raise and set larvae. An extension of the hatchery is the nursery where recently metamorphosed larvae (spat) can be raised to juveniles (about 10 mm shell height for scallops). An integral part of any bivalve hatchery is an algal culture facility where food for larvae, juveniles and adults is raised.

Techniques to breed bivalves in laboratories were established many years ago but it is only in the last 25 years that bivalve hatcheries have produced juveniles on a commercial basis (Loosanoff and Davis 1963, Chew et al 1987). Oyster hatcheries have become particularly prominent on the west coast of North America in the last ten years. It is estimated that over 80% of Pacific oyster Crassostrea gigas seed now planted in North America is derived from hatcheries on the west coast of the United States (Chew, 1988). There are also hatcheries to produce other species of bivalves, manila clams, Tapes philippinarum, hard clams, Mercenaria mercenaria, and common scallops, Pecten maximus.

Hatchery production of seed has several advantages over natural seed collection. Hatcheries can assure a consistent and reliable supply of seed since larvae are raised under controlled conditions. Mature larvae or juveniles can be supplied to the industry, depending on requirements. With proper conditioning of broodstock, seed can be produced at any time of the year. Controlled experimental work can be done at hatcheries and the developmental biology of the animal studied. Genetic manipulation can be carried out at hatcheries, including selection and domestication of strains, and production of polyploid animals. Another potential advantage of hatcheries is that exotic species can be introduced that may be faster-growing and more hardy than native species under culture conditions. Imported species can be held under quarantine conditions in a hatchery, reducing the risk of introducing pests, parasites, and diseases with broodstock. The main disadvantage with hatchery production of bivalve seed is cost, as it is generally much more expensive to produce seed in hatcheries than to obtain it from natural sets.

There are several publications describing the design and operation of bivalve hatcheries but most deal with oysters or clams (eg. Breese and Malouf 1975; Dupuy et al. 1977; Castagna and Kraeuter 1981; Walne 1974). None of these deal with scallops and while the basic technology is similar for most bivalves, there are differences between hatchery rearing of scallops and other bivalves.

The remainder of this publication is devoted to describing the hatchery and nursery technology developed to produce juvenile scallops at the Pacific Biological Station. The primary focus was on two species, the native rock scallop and an imported species, Patinopecten yessoensis, the Japanese scallop (Figure 12).

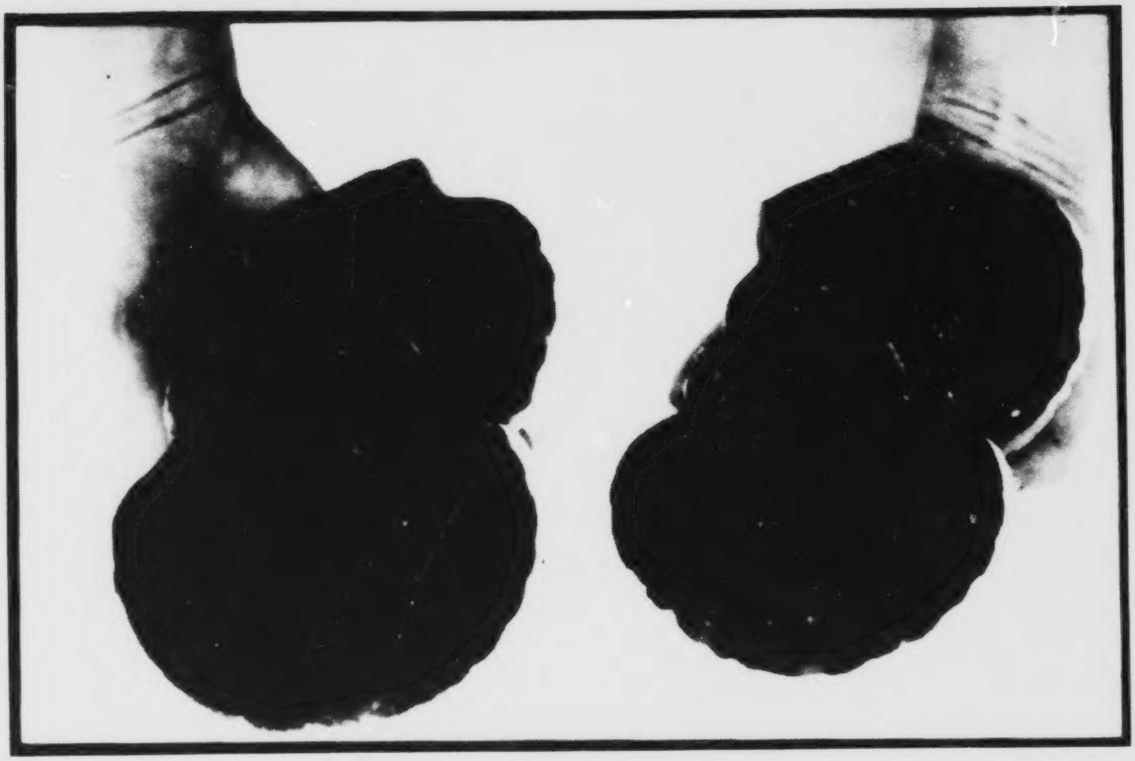

Figure 12. Adult Japanese scallops, 9-10 cm shell height. These animals are F1 generation cultured at the Pacific Biological Station.

3.2.1 Site Selection

Selecting a proper site is probably the most important consideration when locating a scallop hatchery.

It is paramount to ensure there is a good water supply. If this is not available, it will be difficult if not impossible to have an efficient and profitable hatchery operation. No two hatcheries are alike and differences in water quality is often a major contributing factor to varying success.

Pollution, particularly industrial pollution, must be avoided. The lethal and sublethal effects of most industrial pollutants are unknown but can be extremely damaging to bivalve larvae. The recent discovery of the effects of tributyltin (TBT) is an excellent example of this situation (Davies, et al 1986, Davies and Paul 1986). TBT is an anti-fouling ingredient added to marine paints. It is extremely lethal to bivalve larvae, even at concentrations of a few parts per billion. It is advisable to undertake bioassays and water quality tests of water intended for hatchery use before a site is selected. Deleterious materials may be seasonal in nature so sampling should be done over a period of a year.

Scallops have strict physiological requirements, such as water temperature, salinity and oxygen levels, and these must be carefully maintained. Species discussed in this manual are temperate species and water temperatures above 15-18°C can be stressful. Most species occur in oceanic conditions and require salinities above 28 ppt (parts per thousand). Scallops also require water that is well oxygenated. Poor sites for hatcheries generally have high productivity or uncontrollable low salinities from prolonged periods of heavy rains.

If water for the hatchery is pumped directly from the ocean, it is advisable to have intakes below any known thermocline. In the Strait of Georgia, intakes should be more than 20 m below the surface in most areas. Water from such depths has a fairly constant temperature, generally will not exceed 15°C, and is not influenced by fresh water from seasonal rains. Surface waters are subject to seasonal plankton blooms and fouling organisms which can clog filters and may be deleterious to larvae. Care should be taken to avoid supersaturation of any dissolved gases.

In some cases, water for a hatchery can be pumped from saltwater wells and this generally provides high quality water with constant temperature and salinity. In saltwater wells, the water has been filtered through sediment or porous rock and contains few if any fouling organisms. However, it may not be convenient to build a saltwater well and they may be expensive to construct.

The size of pumps and the seawater system in a hatchery will depend on the size of the operation and proposed level of production. It is advisable to allow for future expansion.

All piping should be schedule 40 or 80 polyvinyl chloride (pvc) plastic and all material used must be non toxic to bivalve larvae. If there is any doubt about material it should be bioassayed with standard techniques (Bourne et al 1981).

At the Pacific Biological Station, intakes for the salt water system are about 20 m below the surface of the water at low tide. Temperature of the water ranges from 7.5-14°C and the salinity remains constant at 29 +/- 1 ppt. The water is well oxygenated with no supersaturation of any gases. The water is filtered through sand filters to 70 um to prevent fouling organisms settling in the delivery lines. Approximately 200 l of salt water per minute can be heated to 25°C and the same amount chilled to 2°C.

It is necessary to have heated salt water available to condition broodstock, spawn adults and raise larvae. The amount of heated water required will depend on the size of the hatchery and intended production. Chilled water is needed also for conditioning and holding broodstock. As it is easier to heat than to chill water, a location with cool water temperatures is preferable.

3.2.2 Hatchery Design

Proper design of a hatchery is necessary for efficient operation. Design depends on individual circumstances but it is advisable to thoroughly plan a hatchery before beginning construction.

A hatchery should be as flexible as possible so tanks can be moved to different locations without causing major renovations. Wet lab space is necessary for algal culture, holding broodstock, spawning, tanks to raise and set larvae and tanks for a nursery system. The wet lab area should be provided with large floor drains. There should be a dry lab area where equipment such as microscopes, balances, and coulter counters, can be kept away from salt water. A supply of fresh water is necessary for cleaning and washing. Distilled water should be available for preparing nutrient solutions for algal culture. A compressed air supply is required for both algal and larval culture facilities. Filters to 0.45 um should be put in the air line to remove any impurities that may be toxic. There should be a separate area to contain equipment such as pumps, autoclave, boilers, electrical panels, etc, and also a storage area.

If exotic species or disease studies are contemplated, a quarantine facility is necessary. Effluent from a quarantine facility must be treated to prevent the introduction of harmful organisms to the hatchery or the environment. This necessitates construction of a holding tank where effluent can be treated to destroy any pests, parasites or diseases. The effluent can be sterilized by several methods such as chlorination and ozonation. If chlorination is used, the effluent must be subsequently neutralized before it is released into the environment. For most hatcheries a quarantine facility will probably not be needed.

3.2.3 Algal Culture Room

The algal culture facility is most important and considerable care should be given to its location for convenient and efficient operation. Algae is needed for all phases of culture work and requires approximately one-third of the hatchery area.

Algal culture depends on either natural light, artificial light, or a combination of the two. For natural light, a greenhouse is required, preferably on the south facing side of the hatchery to obtain the maximum amount of sun light. The facility should be located centrally to be convenient to all areas. Size depends on intended production and on method of algal culture used (see Section Algal Culture). Sufficient electrical power should be available for artificial lighting when natural sunlight is inadequate. Compressed air and carbon dioxide will be needed. There should be adequate ventilation to maintain temperatures at or below 20°C on days when bright sunlight heats up the facility. If algae are raised entirely with artificial lighting, it is advisable to locate the algal facility as centrally as possible. The same criteria apply for an outside algal culture facility. Adequate electrical circuits must be provided to operate all the light fixtures needed for culture.

Some hatcheries are two stories in height and the algal facility is placed in the upper room. Algae is supplied to the hatchery by gravity feed.

3.2.4 Larval Culture Room

The hatchery should be flexible since culture methods may change with time. All services, salt water, fresh water, air, electricity should be located conveniently above the tanks but out of the way.

Space is required for holding brood stock, spawning adults, tanks for raising and setting larvae, and initial nursery culture. There should be an area to clean equipment. Actual configuration of the hatchery will depend on personal preference but it is important to carefully consider all the requirements and plan thoroughly before any construction.

3.2.5 Nursery

Nursery operations occur in the hatchery and in the open environment. In the hatchery, animals are held from the time of setting until they are about 1.0 mm shell height. The nursery need not be an integral part of the hatchery but can be located a distance away from it since mature larvae can be readily transported. As discussed later, most bivalve culture can be divided into three phases, hatchery, nursery and growout. A nursery stage, where recently metamorphosed larvae (spat) are grown quickly until placed in the open environment is a most important stage in scallop culture. The nursery will hold animals from the time they metamorphose, at about 0.3 mm shell length, until they are at least 1 mm shell height.

Whether a static or a flow through system is used in the nursery, there should be adequate drains. Natural sea water with an intake about 1 m below the water surface should be available for feeding juveniles, since it is rich in phytoplankton. The water can be filtered through a 50 um bag to remove undesirable algae and possible predators.

The nursery should be located close to the ocean to minimize the length of surface lines, which will probably require cleaning of fouling organisms at regular intervals.

4. ALGAL CULTURE

The algal culture facility is a most important part of a bivalve hatchery. Adequate quantities of high-quality food must be available at all times for successful operations. Scallops feed on unicellular phytoplankton both as larvae and adults and there must be an adequate supply of food to maintain them in a healthy condition. A failure in the algal culture facility can be catastrophic to the hatchery. The manufacture of artificial diets for bivalve hatcheries is still in its infancy. Microencapsulated diets (Langdon et al. 1985; Chu et al. 1987) have been only partially successful in replacing live unicellular algae, which remain the major source of nutrition for filter-feeding organisms (Epifanio 1979; Laing and Millican 1986).

4.1 Species Cultured

Many species of unicellular algae have been cultured and used in bivalve hatcheries. Table 3 shows those species that have been cultured at the Pacific Biological Station. They can be divided into two groups: diatoms and flagellates. All species listed are commonly used in bivalve hatcheries worldwide and are recognized as suitable species in terms of ease of culture and nutritive value.

Some species are suitable as food for different life stages of scallops. For larval stages, only three species are used, a flagellate, *Isochrysis* aff. *galbana* (Tahitian variety), commonly referred to as Tahitian or T. *Isochrysis*, and two diatoms, *Chaetoceros* *calcitrans*, and *Thalassiosira* *pseudonana*. The remaining species, along with T *Isochrysis* and *T*. *pseudonana*, are suitable for nursery culture and broodstock conditioning. Size of an algal cell, presence of spines, or cell wall thickness may preclude use of an algal species as larval food.

4.2 Algal Growth

Growth of unicellular algae is by simple cell division, i.e. a single cell divides to form two cells, which then divide to form four cells, etc. Under normal conditions, an algal culture goes through three phases of growth: lag, exponential and stationary phases (Figure 13). The lag phase occurs when the culture is started and little increase in cell density is observed. In healthy cultures this period is quite short. In the exponential phase cell division occurs rapidly and cell density increases geometrically. Growth is limited only by the time required for cell division in this phase. In the stationary phase, the rate of growth (cell division) declines because some factor, such as nutrients or light, has become limiting and cell density

Table 3. Algal species that have been cultured at the Pacific Biological Station.

Species	Class	Size	Recommended Use
Chaetoceros calcitrans	Baccilariophyceae	2.5-3.75	larvae
C. simplex	"	8.0-9.0	juveniles, adults
C. gracilis	"	5.0-10.0	juveniles, adults
Thalassiosira pseudonana	"	2.5-10.0	larvae, juveniles, adults
Skeletonema costatum	"	6.25-10.0	juveniles, adults
Isochrysis sp. (Tahitian variety)	Prymnesiophyceae	3.25-5.75	larvae, juveniles, adults
Rhodomonas lens	Cryptophyceae	8.0-14.0	juveniles, adults
Tetraselmis sp.	Prasinophyceae	8.0-12.0	(not recommended)

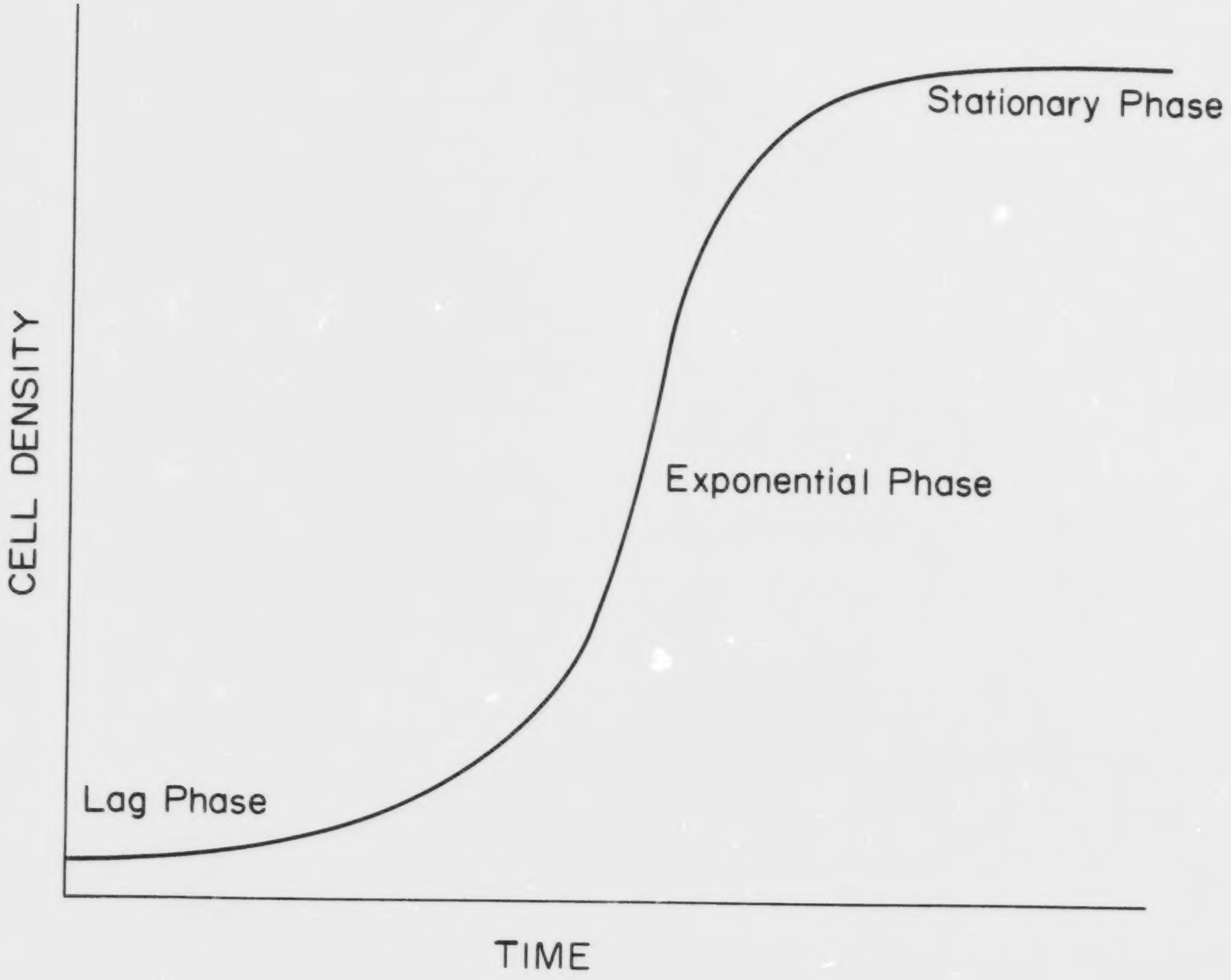

Figure 13. Theoretical growth curve of typical algal culture showing lag, exponential and stationary stages.

remains relatively constant. During these different phases of growth the biochemical content of the algae differs.

4.3 Biochemical Content of Algae

The production of nutritionally sound larvae and juveniles is dependent on the content and nature of biochemical constituents in the algal food. These constituents provide building blocks for tissue formation and metabolism. Physical properties of the algal food can also affect the value of food to an organism. In general, higher food value is obtained from mixed algal diets because they are more likely to contain the diversity needed to satisfy most nutritional requirements for growth and development.

Chemical constituents of algae are rarely constant, and the levels of macronutrients in algae cultured by a specific hatchery will be dependent on the type and content of nutrients used in the algal culture media, the strain of algal species cultured, the conditions of light, temperature, mixing, the phase of growth when collected, and the quality of seawater used to culture the algae. Controlled modification of culture conditions of algae, such as specific nutrient limitation, light and temperature manipulation, have been shown to alter significantly the biochemical composition of the resultant algae (Yoder 1979, Redalje and Laws 1983, Terry et al. 1983, Enright et al. 1986). In general, macronutrient levels in cultured algae and subsequent food value to the target species will be dependent on conditions of growth at the hatchery site.

4.3.1 Energy Content

Some of the more commonly used algae for feeding bivalve larvae have been grown under similar conditions and collected and analyzed for content of macronutrients. The calculated energy, equivalent to the content of these macronutrients is illustrated in Figure 14. *Chaetoceros gracilis* has the most balanced composition of all algae, followed by *Skeletonema costatum*, *Thalassiosira pseudonana*, and *C. calcitrans*. The flagellates, *Tetraselmis suecica* and *Rhodomonas* sp., have an excess of protein, and Tahitian *Isochrysis* an overabundance of lipid, which reduce substantially the content of the other nutrients.

4.3.2 Carbohydrate

Polysaccharides composed of glucose are the principal carbohydrate reserves in the algae (Whyte 1987). In general, diatoms contain a higher level of carbohydrates than flagellates, although the variability in content in the former is dependent on culture phase, particularly in fast growing diatoms such as *T. pseudonana* (Whyte 1987).

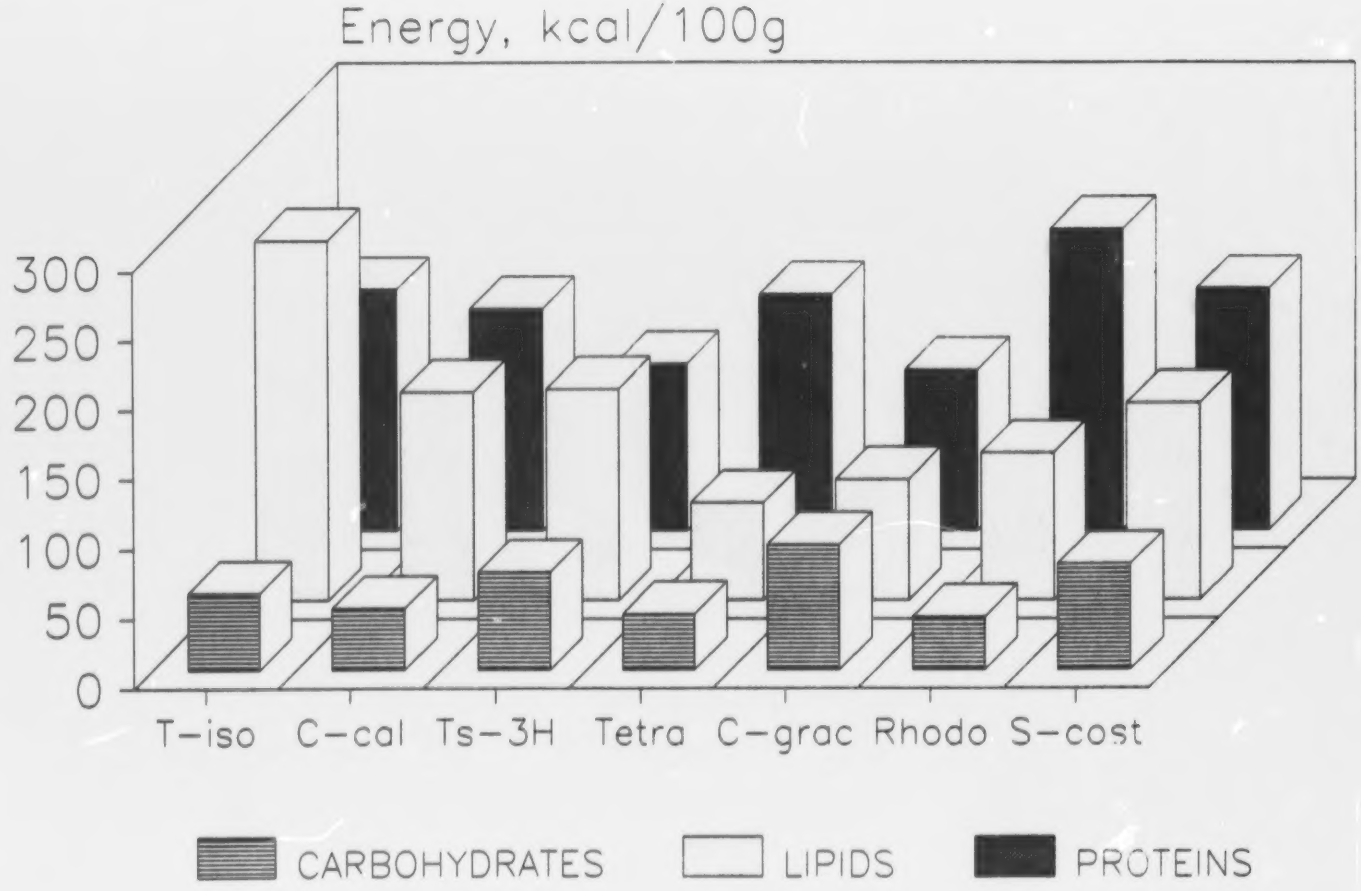

Figure 14. Energy content in kcal/100 g of carbohydrates, lipids and proteins of six algal species used in bivalve hatcheries. T-iso, Tahitian Isochrysis, C-cal, Chaetoceros calcitrans, Ts-3H, Thalassiosira pseudonana, Tetra, Tetraselmis suecica, C-grac, Chaetoceros gracilis, Rhodo, Rhodomonas sp., S-cost, Skeletonema costatum.

4.3.3 Lipid

Percentages of the essential fatty acids in the lipid of some algal species are presented in Figure 15. Tahitian *Isochrysis* is lacking in 20:5*n*3 with less than 1%, but has an adequate level of 22:6*n*3 (11%) to meet nutritional requirements. *C. calcitrans* lacks enough 22:6*n*3 acid, yet has the highest level of 20:5*n*3 in all the algae examined. *T. pseudonana* and *C.* gracilis, together with *Rhodomonas* sp., are all adequate sources of both essential fatty acids. *S. costatum* provides a low level of 22:6*n*3, whereas *T. suecica* has none and would offer an incomplete complement of dietary fatty acids. Complete complements of fatty acids for larval scallops is provided by a mixed diet of any of the diatoms with Tahitian *Isochrysis*.

4.4 Nutritional Requirements of Scallop Larvae

Evaluation of the biochemical composition of mixed algal diets and corresponding mature larvae of Japanese scallops fed these diets, demonstrated that nutritional condition of the larvae was correlated with carbohydrate-rich diets (Whyte et al., in press). Nutritional condition of the larvae was assessed by the total energy derived from the content of lipid, protein and carbohydrate in the larvae (Whyte et al. 1987).

Japanese scallop larvae require a balance between dietary protein to metabolize tissue production, and dietary carbohydrate for metabolism. Higher contents of sugars in the algae allow the amino acids derived from absorbed protein to be saved for tissue formation. Similarly, fatty acids can be used for storage as lipid rather than be degraded for metabolic requirements. The type of fatty acid rather than quantity of lipid appears to be important for adequate nutrition (Figure 15)(Whyte et al. 1987). Therefore, algae with more balanced proportions of macronutrients should be of higher food value to scallops, assuming other nutritional factors are comparable such as, cell size, digestibility, and freedom from toxins or growth inhibitory metabolites.

4.5 Nutritional Requirements of Juvenile and Adult Scallops

The nutritional requirements for scallop spat are still being investigated. The larger sized algae, 10 um or larger, such as *C. gracilis*, *Rhodomonas* sp., *T. suecica*, and the chain forming *S. costatum*, are unsuitable as diets for larvae but may be suitable for juveniles and adults. Based on biochemical composition, *C. gracilis* is potentially the best single algal diet for conditioning broodstock and juveniles.

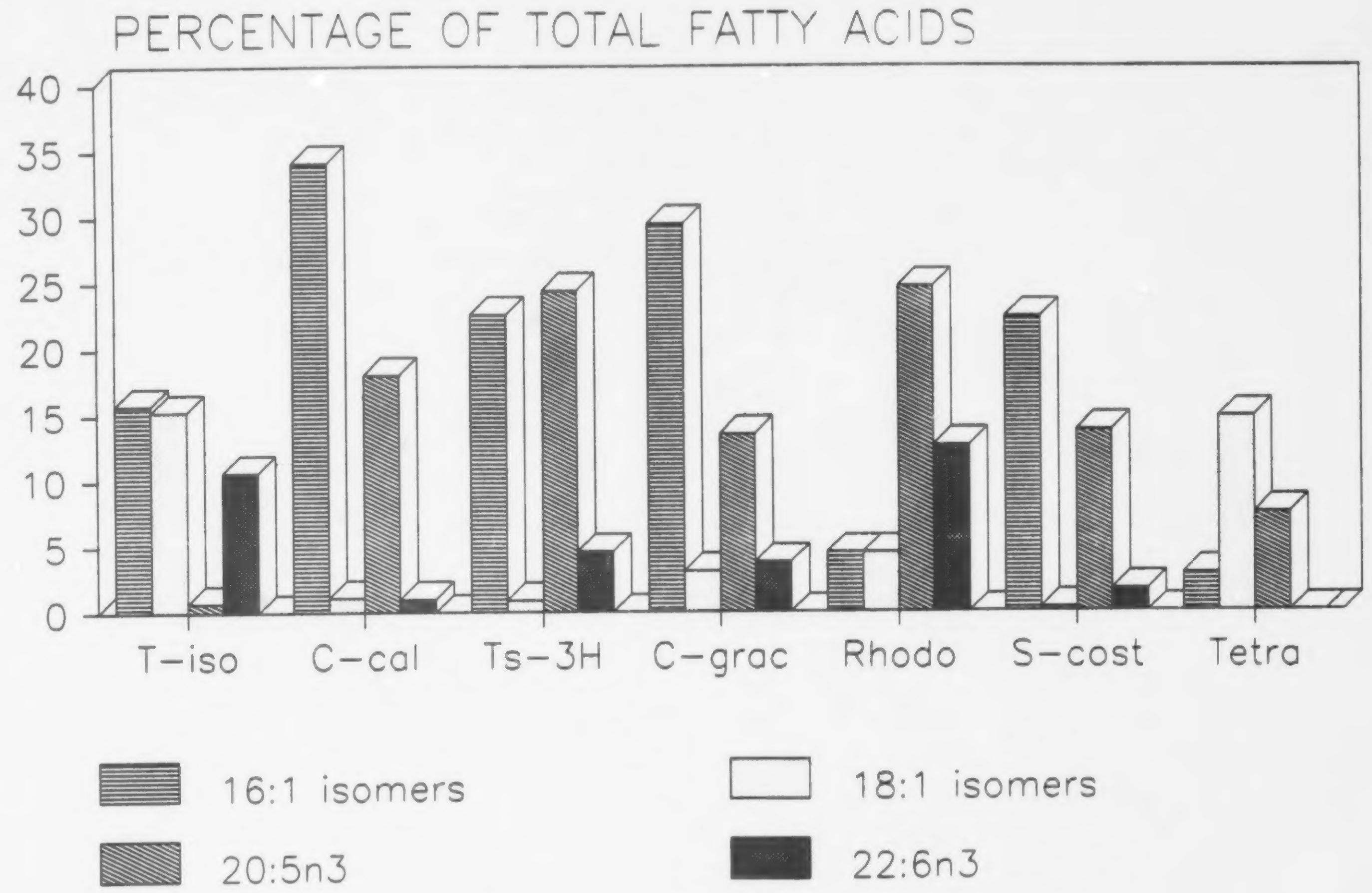

Figure 15. Fatty acid content of six algal species cultured in bivalve hatcheries. T-iso, Tahitian Isochrysis, C-cal, Chaetoceros calcitrans, Ts-3H, Thalassiosira pseudonana, C-grac, Chaetoceros gracilis, Rhodo, Rhodomonas sp., S-cost, Skeletonema costatum, Tetra, Tetraselmis suecica.

4.6 Nutrients

As with all plants, algae must have sufficient nutrients to support growth. Since algae are grown in high concentrations in hatcheries the type and amount of nutrients added to cultures is critical. Several types of nutrient media are available. Two are used at the Pacific Biological Station and these are described in detail. It is advisable to become familiar with a single culture media and use it routinely. If further information on different culture media is desired the reader is referred to the literature.

Different grades (purity) of chemicals are available for the preparation of nutrient solutions. In research laboratories, reagent grade chemicals are used but in many commercial bivalve hatcheries agricultural fertilizers (nitrate, phosphate) are used.

4.6.1 F/2 Media

A standard algal culture media used in culture facilities was developed by Guillard (1975), called F/2 media. It is divided into five parts: phosphate, nitrate, silicate, trace metals, and vitamins (Table 4). For each litre of FSW (filtered seawater), 1 ml each of phosphate, nitrate, silicate and trace metals, and 1/2 ml of vitamin solution is added.

4.6.2 HESAW Media

A more recent nutrient mixture was developed by Harrison et al. (1980), called HESAW (Table 5). This media was developed to support growth of a wide range of phytoplankton. Phosphate, nitrate, and trace metals are combined into a single solution, and silicate and vitamin solutions are the same as for the F/2 Media. One ml of the media is added for each litre of FSW.

4.6.3 Silicate Solution

Silicate solution is added when diatoms (Class Bacillariophyceae) are cultured since silicate is required for formation of the siliceous frustules. When non-diatom species, such as flagellates, are cultured, the silicate solution may be omitted.

4.6.4 Tris Buffer

Tris buffer is required for any culture media that is autoclaved, ie. flask culture (Table 6). The buffer controls pH of the culture media to prevent precipitation of some chemicals during autoclaving. It also buffers the pH of the culture media during algal growth. Two ml of the buffer solution is added to every litre of culture media.

Table 4. Guillard's F/2 media used for culturing algae in bivalve hatcheries.

1.	Nitrate	NaNO3	75 gm/liter
2.	Phosphate	$NaH_2PO_4\cdot H_2O$	5.0 gm/liter
3.	Silicate	$Na_2SiO_3\cdot 9H_2O$	30 gm/liter
4.	Trace Metals		
		$FeCl_3\cdot 6H_2O$	3.5 gm
		Na_2EDTA	4.36 gm

Dissolve in 900 ml distilled H_2O

Add 1 ml of each of the following trace metal solutions

$CuSo_4\cdot 5H_2O$	0.98 gm/100 ml
$ZnSo_4\cdot 7H_2O$	2.2 gm/100 ml
$CoCl_2\cdot 6H_2O$	1.0 gm/100 ml
$MnCl_2\cdot 4H_2O$	18 gm/100 ml
$Na_2MoO_4\cdot 2H_2O$	0.63 gm/100 ml

Bring solution to 1 liter with distilled H_2O (pH ca. 2.0)

Add 1 ml/liter FSW of the above solutions (#1-4).

5. Vitamins

Biotin	1.0 mg
B-12	1.0 mg
Thiamine HCl	20.0 mg

Dissolve in 1 liter distilled H_2O. Store frozen.

Add 1/2 ml of vitamin solution for every 1 liter FSW.

Table 5. HESAW media used for culturing algae in bivalve hatcheries.

1.	$NaNO_3$	466.7 gm
	$Na_2 \cdot glycero \cdot PO_4 \cdot 5H_2O$	66.7 gm

Dissolve in 2 l distilled H_2O.

2.	$Na_2EDTA \cdot 2H_2O$	55.3 gm
	H_3BO_3	38.0 gm

Dissolve in 1 l hot distilled H_2O.

3.	$FeCl_3 \cdot 6H_2O$	1.6 gm

Dissolve in 100 ml distilled H_2O. Add 50 ml to solution #1 and the remainder to solution #2. Mix together solutions #1 and #2.

4.	$MnSO_4 \cdot H_2O$	4.1 gm
	or $MnSO_4 \cdot 4H_2O$	5.4 gm

Dissolve in 50 ml distilled H_2O. Add to above solution.

5.	$Na_2MoO_4 \cdot 2H_2O$	1.26 gm

Dissolve in 50 ml distilled H_2O. Add to above solution.

6.	$ZnSO_4 \cdot 7H_2O$	7.3 gm
	$CuSO_4 \cdot 7H_2O$	1.6 gm

Dissolve in 100 ml distilled H_2O. Add 10 ml of solution to above solution.

7.	Na_2SeO_3	0.173 gm

Dissolve in 1 l distilled H_2O. Add 1 ml of solution to 100 ml distilled H_2O to make stock solution. Add 10 ml stock solution to above solution.

Bring volume of solution to 10 l by adding distilled H_2O. Autoclave prior to use. Add 1 ml of solution for every 1 l FSW.

8.	$Na_2SiO_3 \cdot 5H_2O$	224.0 gm
	or $Na_2SiO_3 \cdot 9H_2O$	300.0 gm

Dissolve in 1l distilled H_2O. Slowly add 1.5 l 1M HCl (133.5 ml concentrated HCl in 1.5 l distilled H_2O). Bring volume of solution to 10 l by adding distilled H_2O. Autoclave prior to use. Add 1 ml of solution for every 1 l FSW.

9. Vitamins

(Follow directions for vitamins in Table 4.)

Table 6. TRIS buffer solution to be added to media that is then autoclaved.

Tris (hydroxymethyl) amino-methane	50.0 gm

Dissolve in 200 ml distilled H_2O. Add approximately 29.3 ml concentrated HCl to bring pH of solution to 7.2. Add 2 ml of solution for every 1 l FSW that is to be autoclaved.

4.7 Culture Conditions

Maintenance of algal cultures depends on several physical parameters. To ensure a consistent and reliable supply of algae, every effort should be made to keep the following parameters as constant as possible.

4.7.1 Temperature

Proper temperature must be maintained to maximize algal growth and temperature fluctuations must be kept to a minimum. Most diatom species that are cultured in bivalve hatcheries, do not tolerate temperatures above 22°C. The flagellate, T. Isochrysis sp., can be cultured at temperatures up to 34°C. Algal cultures should be raised in a temperature controlled room held at 20-22°C, with the exception of stock cultures that are held at 8°C (see below). A temperature recorder or minimum-maximum thermometer should be kept in the algal culture facility to provide a record of temperature variations in the room.

4.7.2 Water

Seawater used for algal culture should be filtered to 1 um. At the Pacific Biological Station, seawater is passed through four cartridge filters, a 10 um, a 1 um, a charcoal filter, and another 1 um filter. The charcoal filter is used to help remove organic material from the seawater. This routine provides seawater of a constant quality.

4.7.3 Light

Light is probably the limiting factor in most algal culture facilities. Adequate light for photosynthesis is required for optimal algal growth. Light limitation can occur because light intensity is too low or the incorrect light spectrum is used.

Light spectrum may affect the growth of algae. Fluorescent lights, cool white, gro-lux, or full spectrum, have been used but no significant difference in algal growth has been reported when cultured under the different types of light. Electrical connections to lights should be protected to avoid contact with salt water. Ballasts for fluorescent lights should be placed outside the algal culture facility to avoid buildup of heat from this source.

For large cultures (3000 l volumes or more) metal halide lamps can be used, but these produce considerable heat. Incandescent lighting is not recommended.

Light intensity can be measured with an inexpensive lux light meter. Light intensity ideally should be approximately 5,000 lux at a distance of 10 cm from the light source, but this is difficult to attain in most hatcheries. If a light meter is not available, have at least one side of the culture container fully illuminated.

Distance from a light source is often the primary cause of light limitation. Light intensities decline sharply as distance from a light source increases, and therefore it is important to place the light source as close to the algal containers as possible. Light limitation can occur because of the shape of the algal container. The difference in light intensity at the side of an algal container that is furthest from the light source will be considerably less than the side closest to the source. An oblong container, rather than a round one, is the most effective way of overcoming light limitation from a single bank of lights. Other ways to overcome light limitation are to immerse a waterproof light in the centre of a round container or to surround the container with lights.

4.7.4 pH (Acidity)

pH is a measure of acidity and this can greatly affect algal growth. As algal cells divide in the culture, metabolites are released into the water which affect pH. Ideal pH levels for algal culture are between 7.2-8.2. A pH of 8.5 or above can be detrimental to the culture and the culture may crash.

The pH can be buffered by addition of carbon dioxide (CO_2). Phytoplankton respire CO_2 as part of their metabolism and therefore can regulate the pH of the culture if a light/dark regime is used. If an algal culture was held in a regime of 12 hours of light and 12 hours of darkness, the pH should remain relatively constant. Small cultures (500 ml) can be regulated in this manner but not large ones. In bivalve hatcheries algal cultures are maintained under a 24 hour light photoperiod to maximize algal growth.

Carbon dioxide gas can be injected into algal cultures along with the air system. A constant level of 2-4% CO_2 mixed with the air supply is sufficient, but the amount required varies with growth rate of the algal culture. In some facilities, CO_2 is injected into algal cultures for 2 minutes every 30 minutes. A more effective way of adding CO_2 into the system is to monitor the pH of algal cultures. A solenoid switch connected to a pH monitor allows CO_2 to enter the air lines when the pH increases above desired levels (Figure 16). When the pH has dropped to 7.2-8.2, the solenoid will close. Such monitors are commercially available.

4.8 Obtaining Algal Stocks

Unialgal and axenic cultures can be obtained from algal collection centres (Table 7). Cultures, generally shipped in 25 ml glass tubes, should be shipped via courier or air express to ensure arrival within two days.

Cultures should be examined on arrival and if a culture is pale in

Table 7. Location of algal collection centers where stock cultures of algae suitable for feeding bivalve larvae can be obtained.

Center for Culture of Marine Phytoplankton (CCMP)
Bigelow Laboratory for Ocean Sciences
West Boothbay Harbor, Maine 04575
U.S.A.
Phone: (207) 633-2173
Telex: 04575 CCMP UD

Dr. Richard C. Starr
Culture Collection of Algae
Department of Botany
University of Texas at Austin
Austin, Texas 78712
U.S.A.
Phone: (512) 471-4019

Northeast Pacific Culture Collection (NEPEC)
Department of Oceanography
University of British Columbia
6270 University Boulevard
Vancouver, British Columbia
V6T 1W5
Phone: (604) 228-4378

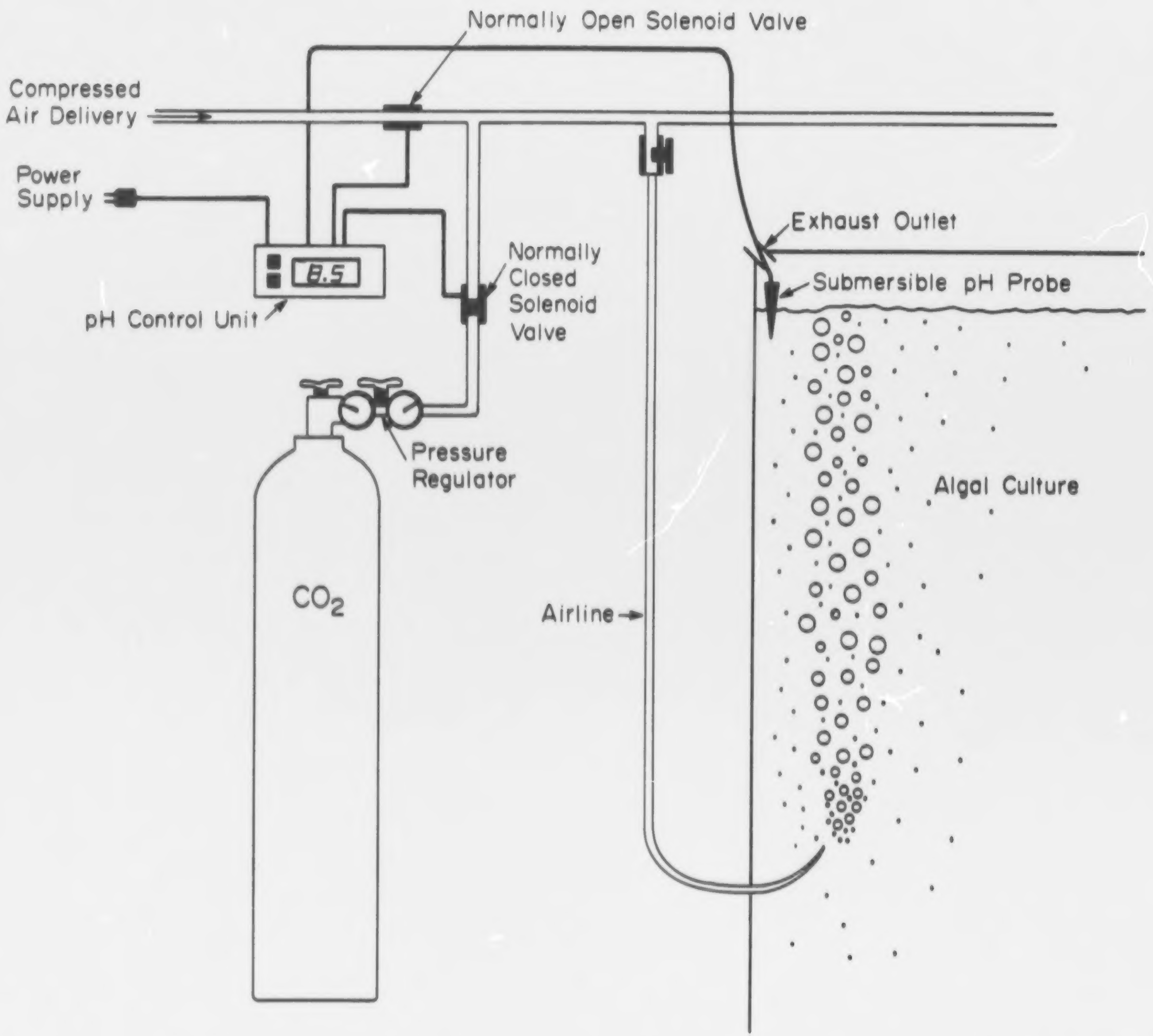
Normally Open Solenoid Valve
Compressed Air Delivery
Power Supply
Exhaust Outlet
Submersible pH Probe
8.5
Normally Closed Solenoid Valve
pH Control Unit
Pressure Regulator
Algal Culture
CO_2
Airline

Figure 16. Diagram of solenoid switch connected to pH meter in algal bag culture system to operate flow of carbon dioxide into algal cultures.

colour, it should be exposed to light for a few days before being transferred (see Section Transfers into Flasks 4.9.2). If clumps or debris are in the culture, it should be transferred immediately. Transfer all but about 5 ml of the culture from the tube to inoculate a 250 ml culture flask. If the transferred inoculum does not grow, a second transfer can be attempted with the remaining inoculum.

4.9 Flask Culture

Flask culture is a convenient method to maintain algal cultures in as clean a condition as possible. Flasks containing FSW and nutrients are sterilized by autoclaving and the media is inoculated with a small amount of unialgal culture.

Erlenmyer flasks generally are used because the shape of the flask allows for easy pouring, yet they have a large base area that allows for maximum surface-to-air interface of the media for gas exchange. Flasks should be no more than 1/2 full at any time. Two sizes of flasks can be used, a 250 ml flask (filled with 125 ml media) for stock cultures and a larger 1 l flask (filled with 500 ml media) for working cultures.

4.9.1 Preparing Flasks

All flasks should be thoroughly cleaned. Algal media is mixed in a carboy with a spigot for easy dispensing. For every litre of FSW, 1 ml of nutrient mix (HESAW media), 1 ml silicate solution, 1/2 ml vitamin solution, and 2 ml TRIS buffer is added. An example is:

Add the following to 20 l of filtered seawater
20 ml nutrient solution (HESAW)
20 ml silicate solution
10 ml vitamin solution
40 ml TRIS buffer

After media is added to the flasks, cotton or foam stoppers are inserted in the neck of the flasks and covered with a double layer of aluminum foil. Cotton stoppers can be made by stuffing cotton batting in a square of cheesecloth and tightly tying the corners together to make a ball. The layers of foil should cover the entire neck of the flask and are intended to prevent airborne contamination of the flask after it is autoclaved.

The flasks are autoclaved to sterilize the media and the inside of the flask. Complete sterilization is effected after 15 minutes at 6.8 kg (15 lb) pressure in a standard autoclave. The autoclave should be returned to room pressure without venting to avoid boiling of the culture media inside the flasks. Flasks should be autoclaved immediately after media is added and be allowed to cool to room temperature before being used. Flasks must be handled carefully and the cotton stoppers should never become wet from splashing of the media inside.

4.9.2 Transfer of Algae into Flasks

Transfer of an algal inoculum into a flask is the time when bacterial contamination is most likely to occur and clinical procedures must be followed. All flask-to-flask transfers should be done inside a transfer cabinet that can be purchased or easily constructed (Figure 17). Before use, the inner surfaces of the cabinet should be washed down with 85% ethanol or a dilute bleach solution. An ultraviolet light should be installed in the cabinet and turned on at least 15 minutes prior to use to destroy any bacteria in the cabinet. Small doors provide minimum opening and allow free movement of hands inside the cabinet. A plexiglass front provides easy viewing. A small burner is used to flame the mouth of each flask during transfers. The procedure for flask-to-flask transfers is given in Table 8.

The lip of a flask should not be touched and flask-to-flask contact should be avoided while pouring. Never place the cotton stopper on any surface, but hold it by the top part of the stopper.

After transfer, the remaining portion of culture in the inoculating flask can be used for starting large cultures or for bacteriological testing.

4.9.3 Stock Cultures

Bivalve hatcheries should maintain stock cultures of all algal species used in their facility in case a failure occurs in the algal lab. Stock cultures are held separately from other algal cultures and should be kept in an incubator away from other algal cultures. Approximately 50 ml of inoculum is used to start a new stock culture flask. The remainder is kept until it is certain that algae in the new flask are growing. If algae in the new flask does not grow, then another flask can be started from the remaining portion. Stock cultures are grown in 250 ml flasks containing about 125-150 ml media and are held at 8°C with 24 hours of light. The cool temperature allows for slower growth and therefore less frequent transfers are needed. Since the flasks are not aerated, they should be swirled gently daily to keep the algae in suspension.

All stock cultures should be transferred routinely, approximately monthly, to maintain them in the log phase growth. If proper care is given to stock cultures, new inocula from algal banks need only be ordered in the unlikely event of contamination or a crash in stock cultures.

The larger 1 l flasks also can be started from the remaining portion of the stock culture. Usually 75-100 ml is sufficient to begin one large flask.

4.9.4 Working Cultures

The larger, 1 l flasks are used as working cultures. They are held at 20-22°C, with 24 hour light, and are transferred routinely every

Table 8. Procedure for transferring algal cultures from flask to flask.

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1. Wipe all inner surfaces of inoculating booth with 85% ethanol.
2. Place all flasks that will be required in the booth ; ie. all flasks to be transferred from (the transfer flask) and flasks containing sterilized media to be transferred into (new flasks).
3. Close booth and switch on ultra-violet lamp. Leave for at least 20 minutes. (It is not safe to look directly at ultraviolet light, so a dark cover should be placed over the plexi-glass viewing plate when the light is on.)
4. Switch off lamp. Ignite small burner.
5. Remove foil caps from one transfer and one new flask. Flame the neck of each flask by slowly rotating the neck through the flame.
6. Tilt the neck of the transfer flask toward the new flask. In one motion, remove both stoppers and pour an inoculum into the new flask. Transfer approximately 50 ml for diatom species and 100 ml for flagellates. Avoid touching the necks of the two flasks. Never touch the portion of the stopper that is inserted into the flask. Once the inoculum is added, replace the stopper in the transfer flask. Slowly flame the neck of the new flask before replacing its stopper.
7. Replace foil cap over the neck of the new flask. Using a waterproof marker pen, label the new flask with the algal species inoculated and the date of transfer.
8. Repeat procedure for all flasks within the booth. Once completed, turn off burner and open booth.
9. Remove all new flasks and place in a well-lit area in the algae culture facility.
10. The remaining inoculum in the transfer flasks can be used to inoculate larger cultures such as 4 litre flasks or carboys.

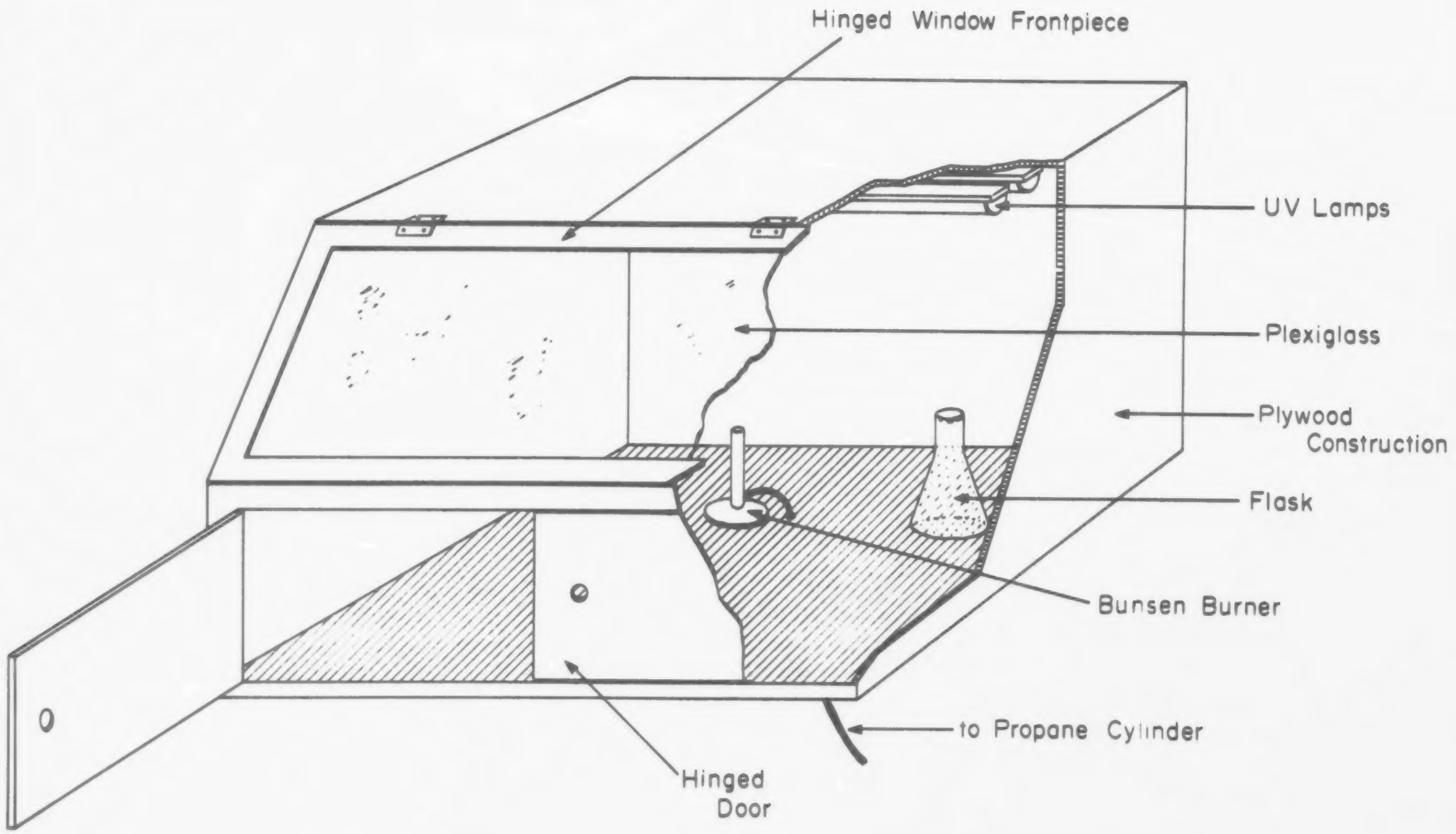
Hinged Window Frontpiece
UV Lamps
Plexiglass
Plywood Construction
Flask
Bunsen Burner
to Propane Cylinder
Hinged Door

Figure 17. Diagram of transfer cabinet used to transfer algal cultures.

second day. The working cultures are not aerated and should be swirled twice daily.

Approximately 100 ml of culture is transferred from the inoculating flask into the new working culture flask. The remainder of the working culture can be used to inoculate larger cultures in carboys. Usually, duplicate flasks of each culture are kept to allow for a sufficient inoculum to start a carboy culture. It also serves as a reserve in the event that algae in one flask does not grow well.

4.10 Batch Culture

Batch culture is a common method used for large-scale algal culture in bivalve hatcheries. Large volumes of algae are grown and harvested once the desired stage of growth or cell density is attained. Each new batch is inoculated from working culture flasks. The method is simple and can be used to culture algae in a variety of containers.

A common culture container is a 20 l glass or polycarbonate carboy. Larger volumes can be produced in clear fibreglass or plastic columns that should be no larger than 0.6 m in diameter since light will not penetrate to the centre of larger diameter columns (Figure 18). In large commercial hatcheries algae can be raised in tanks up to 3 m in diameter and about 1 m in depth.

Seawater for batch culture is filtered to 1 um (FSW) and sterilized by chlorination. A minimum chlorine level of 10.0 ppm for 1 hour is required for effective sterilization (Elston, pers. comm.). At the Pacific Biological Station, a chlorine level of 25 ppm is used. This level of sterilization can be attained by adding 0.5 ml household bleach (5% sodium hypochlorite) for each litre of FSW. Generally, containers used for batch culture are filled with FSW, chlorinated, and left overnight. Containers are filled to the brim so the entire inside of the container is sterilized. The chlorine must be neutralized before the algal inoculum is added and can be done by adding an excess of sodium thiosulphate solution (50.0 mg/l) in distilled water. This solution will dechlorinate an equal volume of household bleach, e.g. if 5 ml household bleach is added to FSW, then 5 ml thiosulphate solution is required to dechlorinate the water.

After addition of sodium thiosulphate, the water should be aerated for at least 30 minutes, to ensure thorough mixing of the thiosulphate and subsequent dechlorination. The aerator, whether glass or plastic, must be sterilized before use, either by putting it in the container when it is being sterilized, or keeping it in a chlorine bath, then rinsing it with hot fresh water before use.

After dechlorination, nutrients and vitamins are added to the FSW. For each 1 l FSW, add 1 ml nutrient mix (HESAW), 1 ml silicate (for diatoms), and 1/2 ml vitamin solution. The algal inoculum is added last. It is important to add sufficient algae to ensure rapid growth of the culture, e.g.

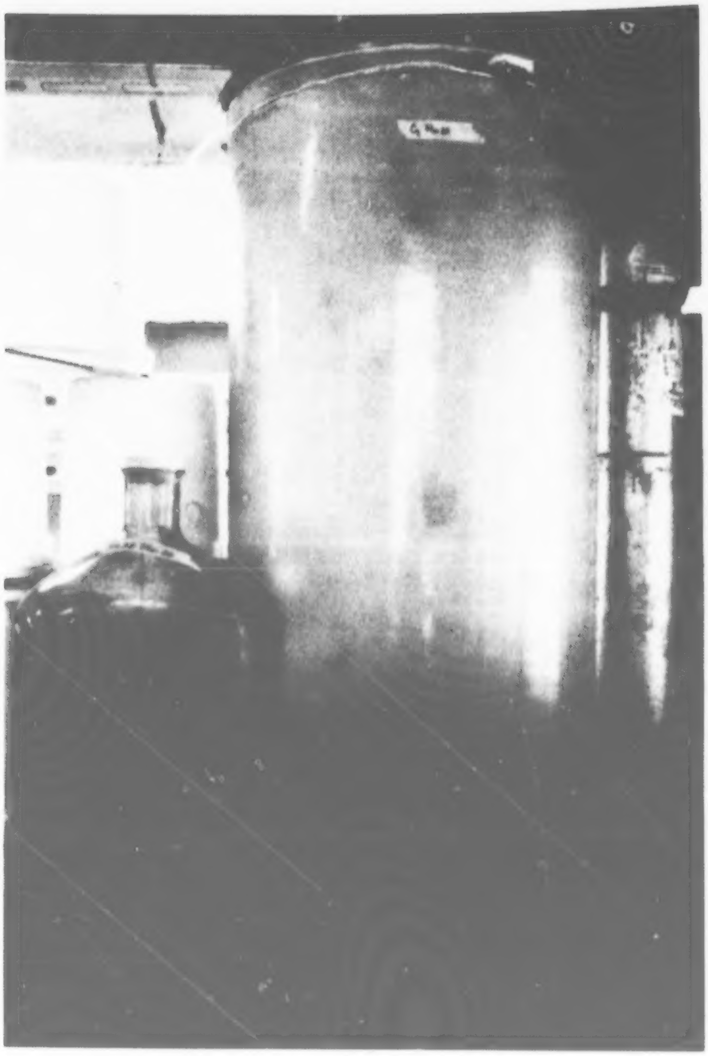

Figure 18. Two methods of algal batch culture used at the Pacific Biological Station, 20 l carboys and 350 l columns.

inoculate a 20 l carboy with 1 l inoculum and a 350 l column with a 10-15 l inoculum.

Batch cultures are kept under a 24 hour light regime at 20-22°C. Harvest of the entire batch occurs when algal cell density has reached the desired level or before the culture crashes, usually 2-6 days after inoculation. The length of time depends on species cultured.

4.11 Semi-Continuous Culture

Semi-continuous culture is a modification of the batch culture system in which only a portion of the culture is harvested at any one time, and the remaining culture is replenished with FSW and nutrients and allowed to continue to grow. A single culture can be maintained for up to 3 months. Eventually, semi-continuous cultures can crash because of a build-up of contaminants, bacteria, or mismanagement. Because a semi-continuous system of culture is kept for a long time, every effort must be made to ensure that contamination from any source is minimized.

4.11.1 Culture Bags

The most common type of container used in semi-continuous culture is a sterile polyethylene bag. They are made of 10 mil polyvinyl plastic and are available in two sizes, 0.5 m x 2 m, and 1 m x 2 m, which hold 250 and 500 l of water respectively. The bags are sealed and the inside is sterile. They can be inflated with sterile air (0.22 um membrane-filtered) to form the shape required before they are filled with sterilized FSW.

The bags must be supported by a frame to maintain a rectangular shape and should be positioned so that the maximum distance from the light source is no more than 20 cm (Figures 19 and 20).

4.11.2 Sterilization of Seawater

Seawater can be sterilized by pasteurization or by filtration through a 0.22 um membrane filter. In the pasteurization process water is heated to 75-80°C, held for a minimum of 30 minutes, and then cooled before it enters the bag. Sterilization by filtration has not been tested on a commercial scale in bivalve hatcheries.

4.11.3 Pasteurization of Seawater

Various types of pasteurizers are used in bivalve hatcheries. The unit at the Pacific Biological Station is described here (Figure 21).

Seawater used for pasteurization is filtered to 1 um and passed through a charcoal filter. When not in use, the heat-exchanger is drained,

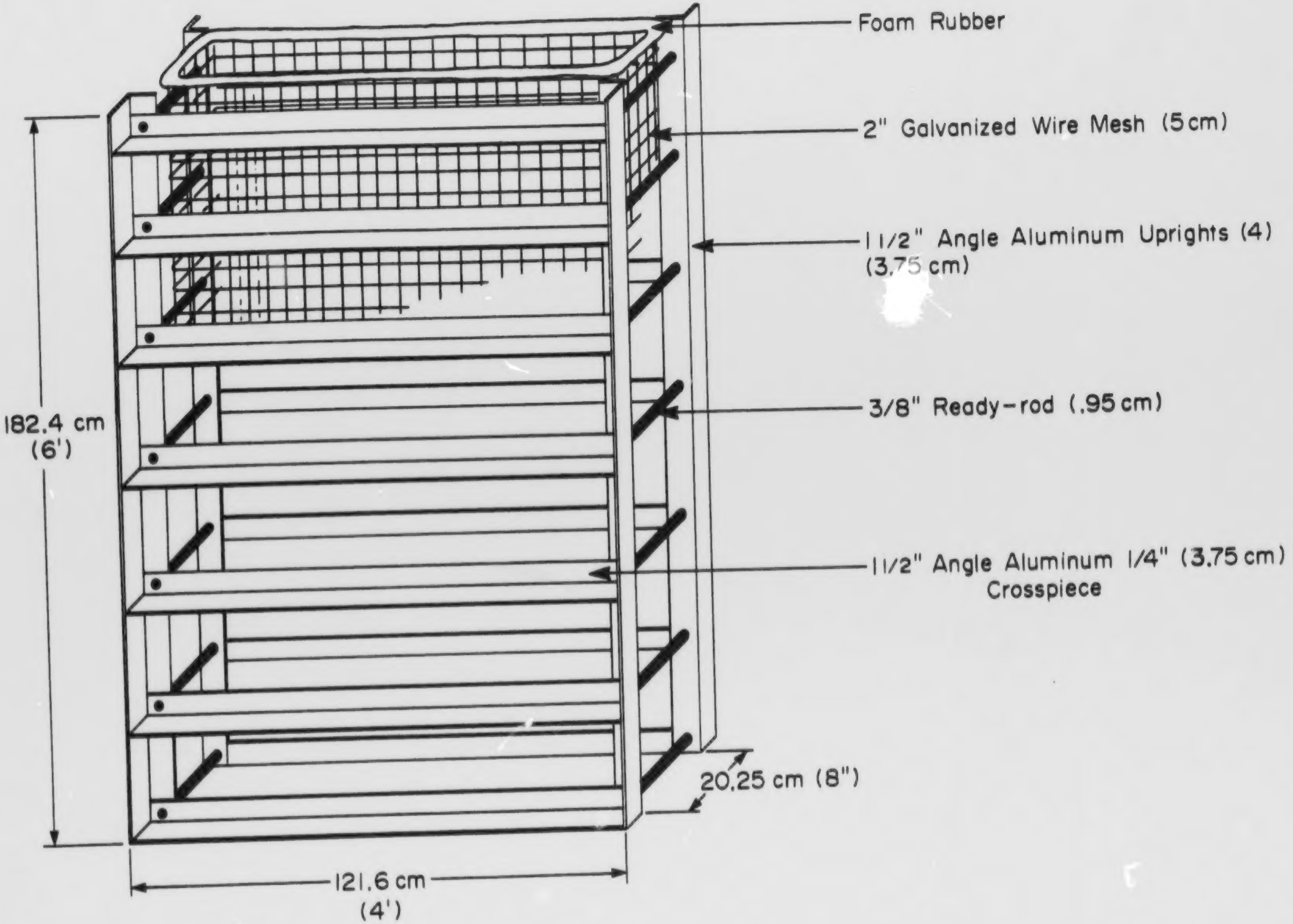
Foam Rubber
2" Galvanized Wire Mesh (5 cm)
1 1/2" Angle Aluminum Uprights (4) (3.75 cm)
3/8" Ready-rod (.95 cm)
1 1/2" Angle Aluminum 1/4" (3.75 cm) Crosspiece
182.4 cm (6')
121.6 cm (4')
20.25 cm (8")

Figure 19. Diagram of frame used to support plastic bags used in algal bag culture system.

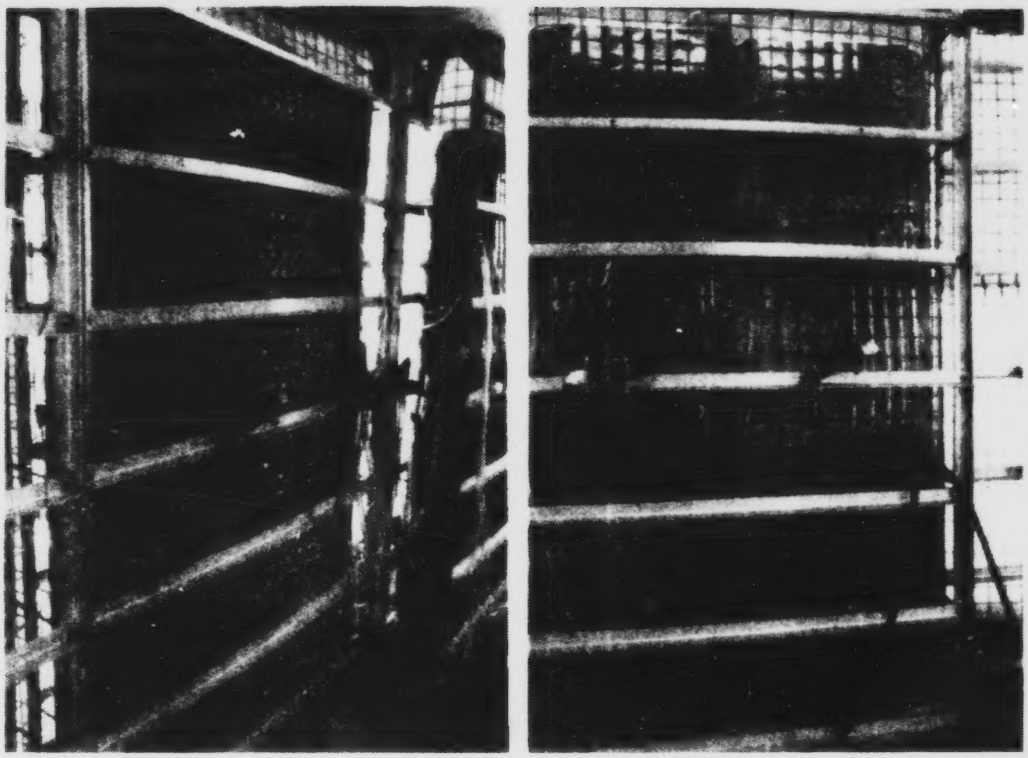

Figure 20. Semi-continuous bag culture at the Pacific Biological Station.

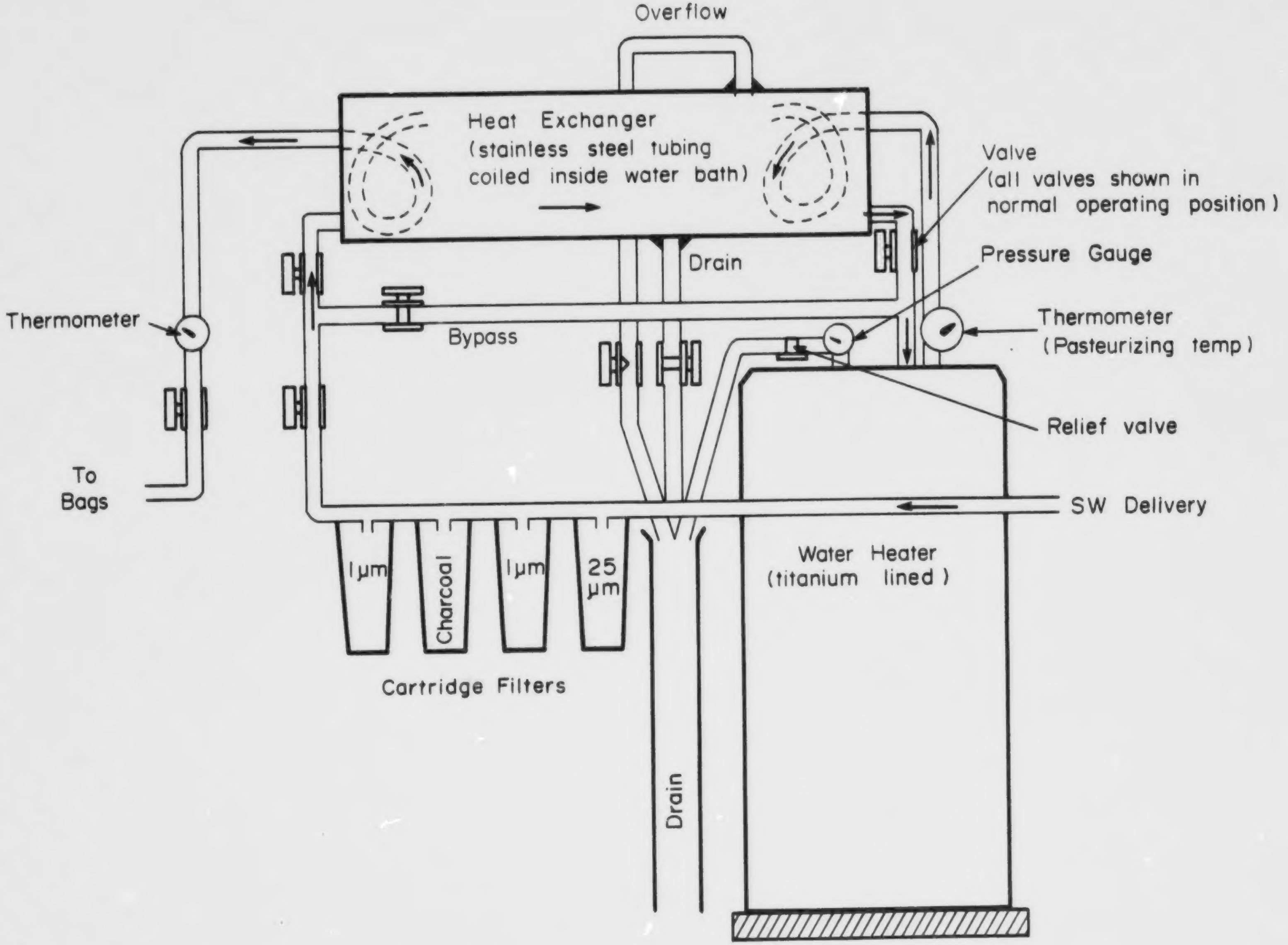
Overflow
Heat Exchanger
(stainless steel tubing
coiled inside water bath)
Valve
(all valves shown in
normal operating position)
Drain
Pressure Gauge
Thermometer
Thermometer
(Pasteurizing temp)
Bypass
Relief valve
To
Bags
SW Delivery
Water Heater
(titanium lined)
1μm
Charcoal
1μm
25
μm
Cartridge Filters
Drain

Figure 21. Diagram of pasteurizer used for salt water sterilization for algal cultures.

and a slow, continuous flow of water is passed through the pasteurizer to avoid stagnation and anoxia.

It is important to ensure that all lines connected with the pasteurizer are sterilized before use. If possible, chlorinated (100 ppm) hot fresh water should be pumped through the system for at least 5 minutes, followed by hot pasteurized seawater for at least 5 minutes before use.

4.11.4 Starting and Maintaining Semi-Continuous Cultures

Clinical procedures must be followed to maintain clean, healthy algal cultures. Airlines or valves inserted into the bag must be autoclaved or rinsed with 95% ethanol, or rubbing alcohol, and the area where they will penetrate the bag also must be swabbed. Compressed air entering bags should be passed through a 0.22 um filter and exhaust openings stuffed with sterilized cotton or foam stoppers to prevent air-borne contamination. Nutrients must be sterilized by autoclaving before use.

The culture bag must be inflated with air before it is filled with pasteurized and cooled FSW to avoid large creases forming in the bag. An air line with a capillary glass pipette affixed to the end is inserted near the bottom of the bag. A 50ml syringe is inserted into each top corner of the bag, one to serve as an exhaust and one for adding nutrients. Nutrients and an algal inoculum are added once the bag is filled with FSW. An advantage of semi-continuous culture is that only a small inoculum is required to start the culture, e.g. 1-10 l is sufficient to start a 500 l culture.

When a bag culture is ready for harvest, a valve is inserted in the lower half of the bag. Cultures are harvested daily or every other day, and refilled with pasteurized FSW and nutrients. The amount harvested should not exceed more than one-third of the total volume of the bag.

A routine should be established to maintain a bag culture in a healthy condition. Ideally, a culture should have the same cell density each time of harvest. This requires the determination of the amount of algae to harvest at any one time, frequency of harvest, and quantity of nutrients required to maintain the culture.

Theoretically, a semi-continuous culture should last indefinitely if all requirements for constant algal growth are maintained. However, some factor ultimately becomes limiting, or the culture becomes contaminated with bacteria. Pasteurization will kill bacteria but not spores, and this can lead to contamination. A semi-continuous culture can last as long as 3 months.

4.12 Continuous Culture

Continuous culture systems monitored by light meters or haemostats have been developed. These systems are not commonly used in commercial hatcheries because they are capital intensive, are very sensitive, and are difficult to install and maintain.

4.13 Monitoring Algal Cultures

Algal cultures should be examined routinely to ensure they are in a healthy condition and to determine when they are ready for harvest.

4.13.1 Visual Inspection

All cultures should be examined daily for clumps or aggregations of cells on the bottom. Colour of the culture is most important and with experience one can quickly determine if a culture is healthy. Possible reasons why cultures are not healthy are summarized in Table 9.

4.13.2 Microscopic Examination

Algae from all cultures, flask, batch, or semi-continuous, should be examined routinely using a compound microscope. Cultures should have cells of uniform size that are not clumped together, and are actively swimming if the species is motile. If the cells are clumped, cell walls broken, more than one species present, or if the culture is badly contaminated with bacteria, it should be discarded.

4.13.3 Estimating Algal Density

Several methods are available to estimate algal cell density, a spectrophotometer, fluorometer, haemocytometer, and coulter counter.

A spectrophotometer or fluorometer measures the chlorophyll A content in an algal culture and this can be used to obtain a quick estimate of cell density. Graphs comparing cell density and readings on either instrument must be prepared for each algal species. However, the chlorophyll A content in an algal cell is not constant and varies with nutritional state of the cell. This will affect the accuracy of cell density estimates derived from the use of these instruments.

More accurate estimates of cell density can be made using a haemacytometer or a coulter counter.

The haemacytometer was developed to count blood cells. It consists of a thick glass slide with two chambers, each 1.0 x 1.0 mm. A special coverslip is placed over these two chambers to give a depth of 0.1 mm so that the total volume of each chamber is 0.1 mm^3. The chambers are divided by a grid to aid in counting cells within the area (Figure 22). Before counting motile algal species, 1 or 2 drops of 10% formalin should be added to a 50 ml sample. The coverslip is mounted over the chambers and one or two drops of the algal sample is placed under the coverslip.

Table 9. List of points to check if algal cultures crash or show poor growth.

---

1. Air supply. Is there adequate air entering the cultures?
2. Temperature. Check min/max thermometer. Were there any increases or decreases in the temperature of the algal culture facility over the past 24 hours?
3. pH. Check CO_2 supply. Is the CO_2 cylinder empty?
   Check pH of the algal cultures using a pH probe. Is the pH too high (above 8.5)? Is the pH too low (below 7.5)?
4. Nutrients. Check records for the last time the cultures received nutrients. This is particularly important for semi-continuous cultures.

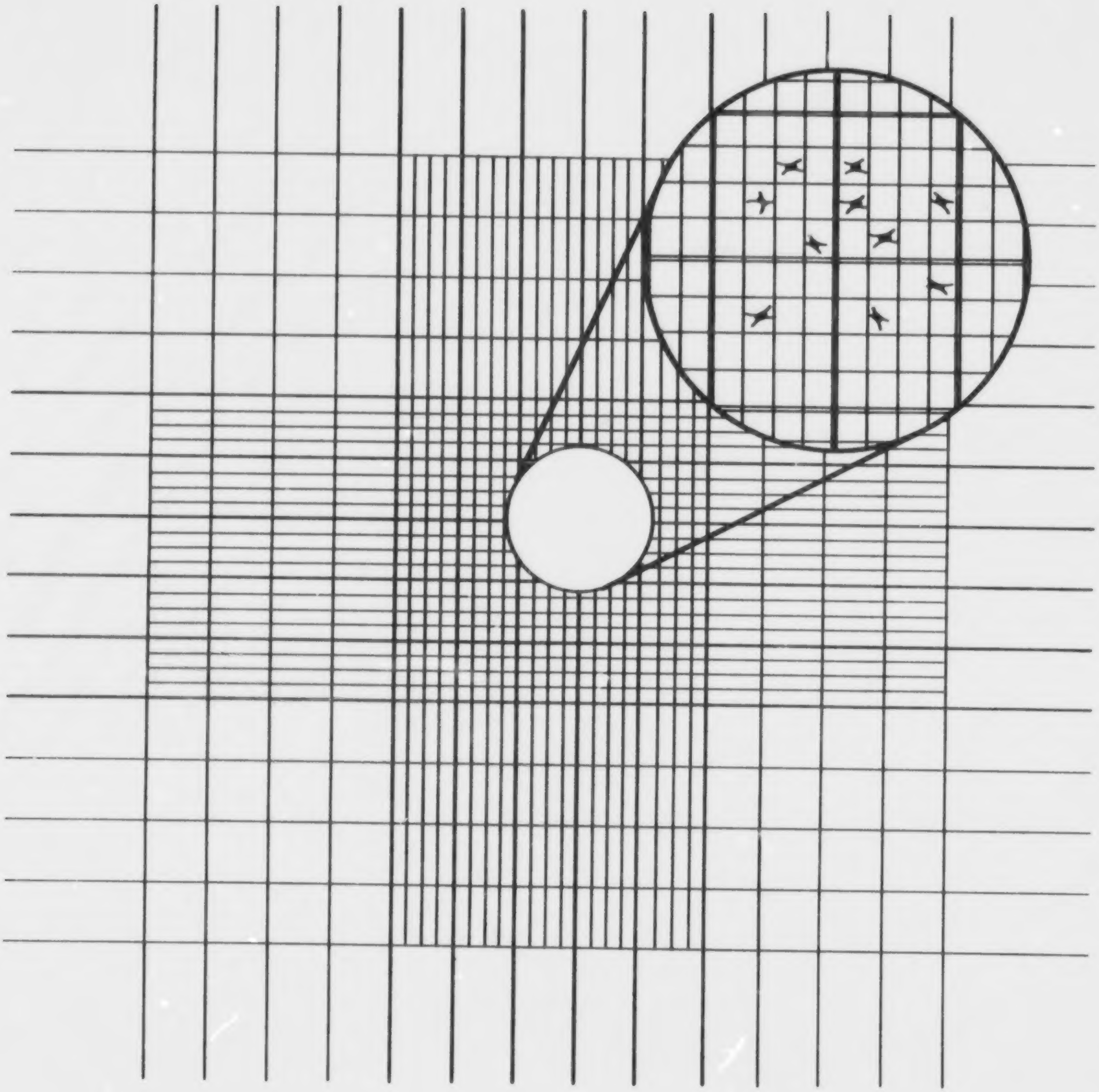

Figure 22. Diagram of counting chamber of haemacytometer used to count algal cells.

Cell density can be estimated by the following method. The central grid is divided into 25 squares, each 0.2 x 0.2 mm, that are again divided into 16 squares, each 0.05 x 0.05 mm. The number of cells in 10 of the 0.2 x 0.2 mm squares are counted and the average or mean is calculated. This gives the mean number of algal cells per 0.2mm x 0.2mm x 0.1mm, or 0.004 mm^3.

The following is an example:

Counts of algal cells: 40 + 30 + 50 + 60 + 55 + 65 + 70 + 45 + 40 + 70 = 525
Average = 52.5 cells/0.004 mm^3
Multiply the average by 250 to give average number of cells per mm^3.
Since there are 1000 mm^3 in 1 ml, multiply the above by 1000.
In this example, the cell density would be:
52.5 x 250 x 1000 = 13.1 million (13.1×10^6) cells/ml.

An easier and more accurate method of estimating cell density is to use a coulter counter (Figure 23). This instrument was also developed for counting blood cells.

Several models are available and all operate on the same principle. A small electrical current is established between two points. Each time a cell passes between them, the current is broken and the cell is counted. The size of the aperture tube is important, and for counting algal cells between 2-10 um an aperture of 50 or 100 um in diameter is required. A known volume of water is drawn through the opening in the aperture tube and the cells are counted. More detailed explanations on the operation of a coulter counter are available (Sheldon and Parsons 1967, Kranck and Milligan 1979).

Since algal cultures are often dense, samples must be diluted to a density that can be counted accurately, approximately 50,000 cells/ml. Algal samples are usually diluted with a 3% solution of sodium chloride (table salt).

The following is an example:

Add 0.2 ml of algal culture to 20 ml 3% NaCl. Mix well. Count 3 times and obtain a mean value.
eg) 5280, 5336, 5120
If the volume of the solution sampled by the coulter counter is 0.1 ml, then the average = 5245 cells/0.1 ml.
To determine the number of cells in 1 ml of the
original algal culture, multiply 5245 by 10 to obtain the number of cells in 1 ml of the sample, and multiply by 100 to correct for the dilution factor.
In this example, cell density would be:
5245 x 10 x 100 = 5.2 million (5.2×10^6) cells/ml.

Coulter counters are expensive but used machines can be purchased

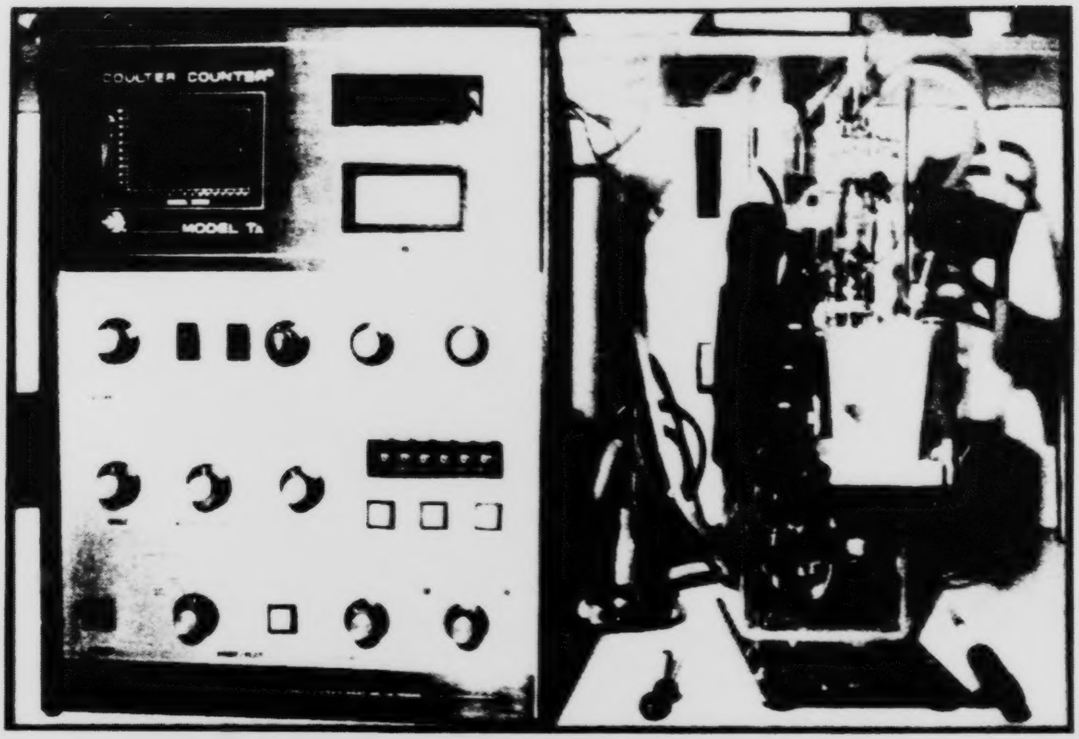
COULTER COUNTER®
MODEL TA

Figure 23. Coulter Counter used to count algal cells and particles in seawater.

for a reasonable price. The cost of purchase is quickly offset by time saved and the accuracy of the counts.

4.13.4 Bacterial Contamination

Bacterial contamination generally is the most common cause of problems or failure in algal cultures. Algal cultures used in bivalve hatcheries are not axenic (bacteria-free). Bacterial levels in algal cultures must be kept under control since they can depress growth of the algae and cause cultures to crash before reaching harvestable densities. Bacteria may actually compete with algae for nutrients (Mathiesson and Toner 1966). Algal cultures that have high levels of bacteria may be toxic to bivalve larvae even though the algal cells appear healthy (Calabrese and Davis 1970).

All flask cultures should be examined monthly using a compound microscope to ascertain levels of bacteria. Batch or semi-continuous cultures should be examined daily to avoid using contaminated cultures. Cultures with high levels of bacterial contamination, e.g. 1:1 ratio of algal cells to bacteria, should not be fed to larvae but should be discarded or fed to broodstock or late juveniles. If a severe bacterial contamination occurs in stock cultures, every effort should be made to clean the culture with antibiotics (see below) or a new culture should be ordered from an algal culture centre.

4.13.5 Bactopeptone Testing

Bactopeptone testing is a method that can be used to determine levels of bacterial contamination in algal cultures and need only be done for stock flask cultures. To prepare bactopeptone media, dissolve 10 gm bactopeptone in 1 l FSW. Fill test tubes with 10 ml of media, cap the tubes, and autoclave. Tubes containing the bactopeptone media are inoculated with 3-4 drops of algal culture and stored in the dark for up to two weeks. Some faster-growing bacteria will become evident within 2-4 days. If bacteria are present, the contents of the tube will appear cloudy. Select algal cultures that have the least amount of bacterial contamination, as evidenced by the degree of cloudiness in the bactopeptone test, and discard the remainder.

4.14 Antibiotic Treatment of Algal Stocks

Antibiotic treatment generally is used only for stock flask cultures because it is labour intensive and is done whenever bacterial contamination is identified. If serious contamination is observed, it may be more economical to simply order new algal cultures from an algal culture centre. A method to help prevent bacterial contamination is to treat stock cultures with antibiotics on a regular basis (4 times yearly).

The method used for treating stock cultures is described by Stein (1973) and involves use of penicillin G (potassium or sodium salt), streptomycin sulphate and chloramphenicol. However, experience shows that chloramphenicol can be toxic to the algae. The antibiotic solution used at the Pacific Biological Station consists of 100 mg penicillin G (potassium or sodium salt) and 50 mg streptomycin sulphate dissolved in 10 ml distilled water and passed through a 0.22 um filter into a sterile septum bottle. The solution may be stored at 5°C for up to 5 days. Add 4 ml of the antibiotic solution to 250 ml flasks containing 100 autoclaved media and add 25 ml algal inoculum (see Section Transfers into Flasks for procedure). Place flasks under constant light at 19-22°C for 24 hours. Transfer 25 ml of the media from the treated flask to a flask containing sterile media without antibiotics. To ensure that the bacterial contamination has been reduced to an acceptable level, a bactopeptone test should be done.

5. HATCHERY

The goal of the hatchery is to produce quantities of juvenile scallops by conditioning and spawning adults, and rearing larvae and juveniles. Each part is essential to the success of the hatchery and routine procedures must be followed closely if adequate production is to be achieved.

At the Pacific Biological Station, hatchery work has focused on two species, the native rock scallop and an exotic species, Patinopecten yessoensis, the Japanese scallop. Japanese scallop broodstock was imported from Mutsu Bay, Aomori Prefecture, Japan, 1983-1986, and was held and spawned under quarantine conditions. Since the program has been successful and adult Japanese scallops have been produced in British Columbia, broodstock importations are no longer required.

The remainder of the manual is compiled from technological developments made in the culture of these two species. Equipment utilized in the hatchery or nursery are listed in Appendix 1, followed by suggested suppliers.

5.1 Broodstock

At the Pacific Biological Station, no attempt was made to induce the gametogenic cycle in the broodstock, but only to enhance the final stages of the cycle. Broodstock was brought into the hatchery approximately 3-5 weeks prior to the desired time of spawning. This must coincide with the natural gametogenic cycle so adults are entering spawning condition or are already ripe. By holding them in a controlled environment, the spawning window of the species can be extended over several months. Generally, manipulating holding temperatures and amount of food fed to broodstock can control the period of spawning.

In Japan, the gonad condition index (wet weight of gonad over total wet weight of soft parts x 100) is followed to determine when the animals are sexually ripe (Ventilla 1982). With experience, it is possible to determine when most animals are in spawning condition by visual observation.

5.1.1 Care and Conditioning

Procedures for conditioning broodstock vary with species. Japanese scallops spawn in Mutsu Bay when water temperatures increase to 5-6°C, mid-February to end of April (Ventilla, 1982). Japanese scallops held in Departure Bay appear ripe by early January and spawning probably occurs when water temperatures begin to warm from a winter low of about 7°C. Animals brought into the laboratory in early January can be conditioned to spawn within two weeks by holding them at 7-8°C and feeding them a continuous supply of algae (about 10-15,000 cells/ml). Broodstock held under these conditions can be spawned from early February to the end of July.

Rock scallops have a protracted spawning period from June to September in British Columbia waters (Bourne, 1988). Adults brought into the laboratory as early as May can be conditioned for spawning by holding them at 12-15°C and feeding them continuously. In 1986, broodstock was conditioned and spawned from June to January.

Once the animals are in spawning condition, the water temperature should be dropped 2-4C° to avoid accidental spawning of the broodstock in the holding tanks.

Broodstock are held in shallow tanks supplied with a constant flow of seawater. Care should be taken to ensure tanks are not overcrowded, no more than 2/3 of the bottom area is occupied by animals. They should not be stacked on top of one another. Many species, e.g. Japanese scallops, often shift position presumably to distance themselves from one another. This may cause undue stress and unnecessary expenditure of energy. Rock scallops do not move and can be placed in the tank so that they are not touching one another.

An adequate flow of water must be maintained to ensure adequate dissolved oxygen levels, e.g. about 10 l/min per 100 l of water. An air stone can be placed in each tank to help maintain dissolved oxygen levels and assist in maintaining food particles in suspension.

A recirculating pump may be used to augment flow rates of incoming water. An advantage to using a recirculating pump is that flow rates are enhanced without requiring larger volumes of water, and hence algal food will remain in the tank for a longer period of time. There is a greater likelihood of the food being consumed by the broodstock before being flushed away.

Broodstock tanks should be inspected daily to ensure incoming flow rates and food supplies are adequate. Moribund animals, i.e. animals with retracted mantles, gaping, and not responsive to touch, should be removed immediately. Tanks should be cleaned routinely, at least once a week, to remove faeces and dead algae since their accumulations can produce undue stress on the animals.

Broodstock tanks should be supplied with a constant food supply by pumping algae directly from the algal room (Figure 24). Algae is pumped every 30 minutes from a large algal culture to a header tank (approximately 50 l volume) and gravity-fed to each broodstock tank. Usually a mixture of species is fed (10,000-15,000 cells/ml)(Table 3). The actual volume of algae required to feed the broodstock depends on the number and size of adults being conditioned, the density of the algae and its residence time in broodstock tanks. Generally, 300-700 l of algae is fed to 100 broodstock animals daily.

Food lines must be cleaned regularly to avoid a build-up of bacteria and dead algal cells. This can be done by passing chlorinated water through the lines on a regular basis, and allowing the water to go to waste. To avoid a rapid buildup of material, food lines can be periodically flushed

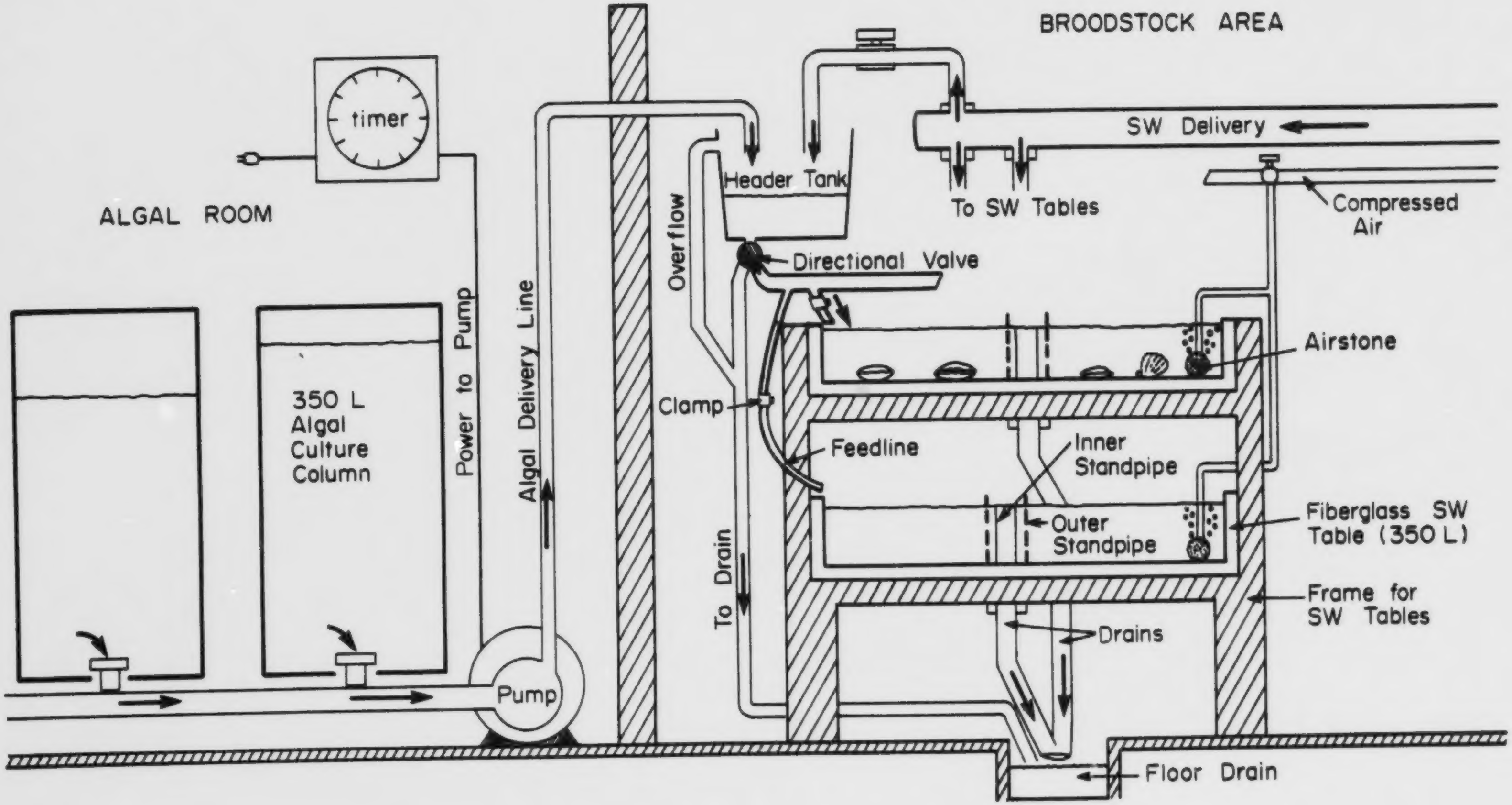
BROODSTOCK AREA
ALGAL ROOM
timer
Power to Pump
350 L
Algal
Culture
Column
Pump
Algal Delivery Line
Overflow
Header Tank
Directional Valve
Clamp
To Drain
Feedline
SW Delivery
To SW Tables
Compressed
Air
Airstone
Inner
Standpipe
Outer
Standpipe
Fiberglass SW
Table (350 L)
Frame for
SW Tables
Drains
Floor Drain

Figure 24. Diagram of continuous feed supply system for broodstock tanks.

with clean seawater between pulses of algae with a slow, constant flow of seawater entering the header tank.

5.2 Spawning

Adults used for spawning should be placed in a separate tank one or two days prior to spawning. Generally, 10-20 females and 5-10 males are selected for a single spawning. Broodstock intended for spawning should have large, plump gonads and have been conditioned for at least 3 weeks. Initially, it may be necessary to make quantitative assessments of the gonad to determine gonad ripeness. This can be done by measuring the gonad condition index, wet weight of gonad over wet weight of total soft parts x 100 (Ventilla, 1982). A gonad index of 25-30% is desirable for successful spawning. Another method is to prepare histological sections. However, these methods require the sacrifice of a large number of animals. With experience, it is possible to determine gonad ripeness by visual inspection.

Gonads of Japanese scallops can be easily observed because the valves can be opened readily. Rock scallops are more difficult to open without damaging the animal. They may be stimulated to open by inserting a small strand of stiff nylon line into the mantle cavity through the byssal notch gap. Once the valves gape slightly, a blunt instrument can be used to pry the valves open further. When sex and gonad ripeness have been determined the animals should be marked to avoid repeated opening.

When examining broodstock, care should be taken not to damage the animal. If too much pressure is applied when opening the valves, the hinge ligament can be broken, or the adductor muscle can be torn away from the shell. The mantle tissue should never be compressed against the shell.

Seawater used for all phases of spawning should be filtered to at least 5 um. Broodstock should be scrubbed clean of epifauna.

Several methods can be used to spawn bivalves (Loosanoff and Davis 1963, Matsutani and Nomura 1982). The two most frequently used methods to induce spawning in scallops at the Pacific Biological Station are thermal shock and injections of serotonin into the adductor muscle.

5.2.1 Thermal Stimulation

Thermal stimulation is the common method of spawning bivalves and should be tried before other methods are used. It will generally induce spawning of animals when gonads are fully ripe.

Approximately 18 hours before spawning Japanese scallops, water in selected broodstock tanks is gradually warmed from the holding temperature (8°C) to 10-11°C. Broodstock are removed from the water, exposed to air (15-18°C) for 1-1.5 hours and re-immersed in 14-16°C, UV-irradiated seawater. Males generally will start to spawn within 1 hour after re-immersion and females will follow soon after. The release of sperm into seawater frequently will stimulate other animals to spawn.

Japanese scallops can be spawned by thermal stimulation from February to June. After June, it may be more difficult to maintain broodstock in a ripe condition and other methods must be used to induce spawning.

Thermal stimulation can also be used to spawn rock scallops, but they should be re-immersed into 18°C UV-irradiated seawater.

5.2.2 Serotonin

If thermal shock does not induce spawning, an alternative common method is an injection of 0.2 ml of 2 x 10^{-4} M solution of serotonin creatinine sulphate (8 mg dissolved in 10 ml filtered seawater) (Matsutani and Nomura, 1982). The solution usually is injected into the adductor muscle, although it can be injected into the gonad. Once serotonin is injected, the animals clap their valves rapidly for 30 seconds to 1 minute and then rest with the valves gaping slightly, sometimes with the foot extended. Depending on ripeness of the gonad, spawning can occur within 10-30 minutes after injection.

5.2.3 Other Methods

Other methods to induce spawning have been used with some success. Additions of large quantities of algae or sperm suspension to the spawning tray can trigger spawning. Sudden chilling of the water to 2-5°C followed by sudden warming will sometimes cause spawning. Additions of potassium chloride (KCl) or hydrogen peroxide are also known to stimulate spawning in bivalves (Morse et al. 1977), but they were not successful in spawning scallops at the Pacific Biological Station.

5.2.4 Spawning Procedures

When spawning begins, the scallops should be removed to a separate container and allowed to continue to spawn. Usually, 4-5 spawning females or males are placed into a 15-l glass jar filled with FSW (14-15°C). Males and females are kept separate. Females should be rinsed with FSW before transfer to a separate container to avoid contamination by sperm.

Copious amounts of sperm are usually released during spawning and very little is required to fertilize the eggs. Males are allowed to spawn in a jar until the water becomes cloudy and then are transferred to another jar. Only 1-2 l of sperm suspension is saved and the remainder discarded. Since there is usually a lag between the time when males and females spawn, the sperm suspension is kept cool in a 12°C water bath. As the males continue to spawn, the most recently spawned suspension is used for fertilizing eggs.

Females are allowed to spawn in a jar until the water becomes cloudy with eggs (10-60 minutes) and then transferred to a fresh jar. Eggs should be fertilized soon after release, no later than 1 hour after release.

Generally, spawning can be stopped by placing the animals in chilled seawater (2-3°C), and they can be recorditioned for further spawning.

5.3 Fertilization

After females have been removed from the jar, the egg suspension is fertilized by adding a small quantity of sperm suspension. The egg suspension is constantly agitated while approximately 20-50 ml sperm suspension is added to a 10 l volume of eggs. After 10 minutes, a sample of eggs is examined under a dissecting microscope. If there are 3-4 sperm attached to the outside of each egg, the sperm-egg ratio is adequate to ensure good fertilization. If the ratio is lower, more sperm can be added.

5.3.1 Rinsing Eggs

Within 20 minutes of fertilization, the eggs are passed through a 105 um screen, to remove debris or large clumps of eggs, and collected on a 35 um screen. The eggs are rinsed with FSW while on the smaller screen to remove additional debris and excess sperm. The smaller screen should be submerged in a tray of water so that the eggs are not pressed against the screen as they are collected.

5.3.2 Collecting Eggs

After the fertilized eggs are rinsed, they are resuspended in a known volume of FSW and a sample taken to estimate numbers. The eggs are evenly distributed in the water by mixing in an up-and-down motion with a plunger (Figure 25). Eggs should not be stirred or they will concentrate in the centre of the container. While mixing with the plunger, a 1 ml sample is taken with a pipette. The sample is placed on a Sedgwick-Rafter counting cell and the eggs counted under a dissecting microscope. To avoid double-counting eggs the area of the counting cell should be divided into columns (Figure 26). A mechanical counter is useful for recording the number of eggs on the slide. From the 1 ml sample, the total number of eggs in the egg suspension can be determined by multiplying the number of eggs in the sample by the total volume of the egg suspension in mls.

5.3.3 Abnormal Fertilization

Abnormal fertilization may be caused by several factors. Too many sperm added to the egg suspension can cause polyspermy, when more than one sperm cell penetrate the same egg membrane, and this causes irregular development. Eggs fertilized more than one hour after release are likely to develop abnormally. Work with oysters has indicated that the longer the period the eggs have resided in a female after becoming mature, the higher the

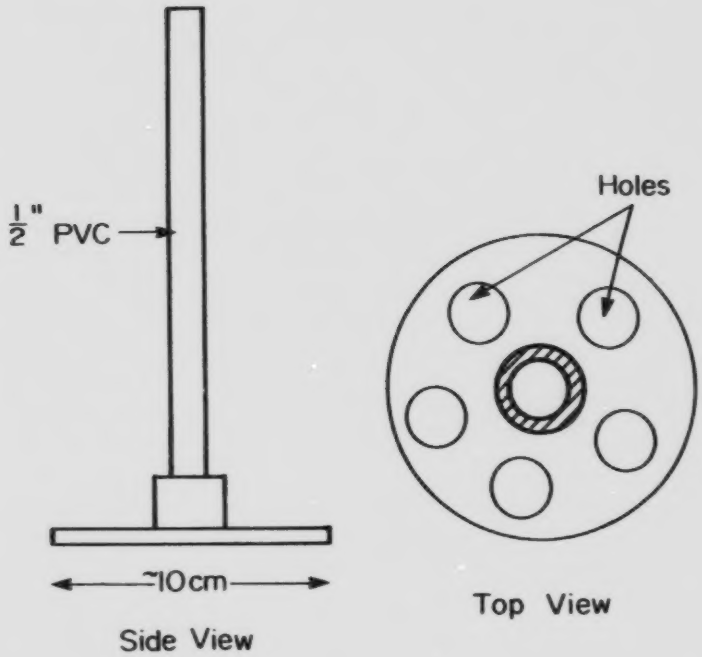
$\frac{1}{2}$" PVC
~10 cm
Side View
Holes
Top View

Figure 25. Diagram of plunger used to thoroughly mix larvae in a container prior to sampling.

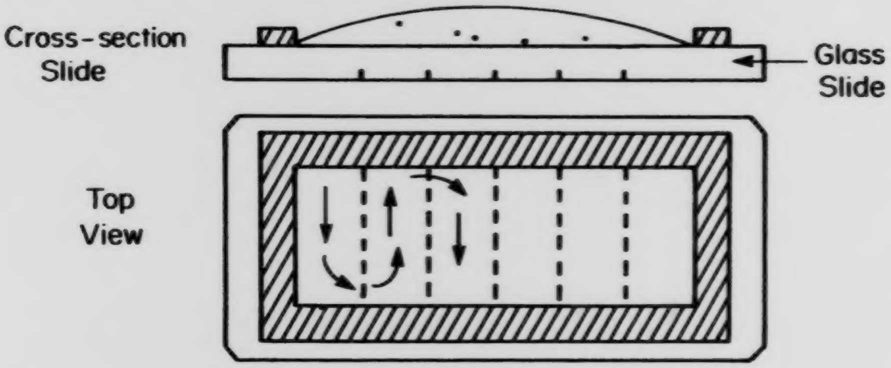
Cross-section
Slide
Glass
Slide
Top
View

Figure 26. Diagram of Sedgwick-Rafter cell used to count larvae and estimate total larval numbers. Arrows indicate direction of scanning the slide.

incidence of abnormal development. A similar phenomenon may occur with scallop eggs.

5.4 Embryonic Development

After the number of fertilized eggs (embryos) is calculated, the egg suspension is poured carefully into a tank filled with FSW. Experimental work done in Japan shows that optimum embryonic and larval development of the Japanese scallop occurs at 10-15°C and 30-40 ppt (Yamamoto 1968). Experimental work at the Pacific Biological Station has shown that optimum embryonic development for the Japanese scallop occurs at 29 ppt and 15-18°C (Figure 27, Table 10). Rock scallop embryos develop at 25-29 ppt and 15-18°C (Figure 28). Scallop embryos are heavier than water and sink to the bottom of the tank. Only a monolayer of embryos should be in a tank, or about 50 million eggs in a 2.5 m diameter tank. The water should not be aerated or the embryos will collect in windrows at the edge of the tank because of water circulation.

Embryos can be left for 72 hours after fertilization, during which time they develop through the trochophore stage and into veliger larvae. During embryonic development, the animals are fragile since they are soft-bodied and can be easily damaged if collected on screens. After embryonic development is completed, the resulting veliger larvae can be collected on screens without causing severe damage.

5.5 Larvae

Larvae are reared in tanks containing FSW that is gently aerated. Size of tank will vary with need, but generally the larger the tank the better. Surfaces tend to have higher numbers of bacteria and so it is preferable to minimize the surface area to water volume ratio. Rearing temperatures differ for various species; for Japanese scallops it is 15°C and for rock scallops it is 18°C. Aeration of the tanks should not be vigorous, just enough to continually mix the water. The air stone should be suspended about 10 cm above the bottom to prevent mixing of dead or diseased larvae or detrital material that may accumulate on the tank bottom.

Larval tanks should be positioned so they are not subjected to direct sunlight. The tops of the tanks can be covered with dark plastic to block out direct sunlight and also prevent debris that may be toxic or bacteria-laden from falling into the tanks. Direct sunlight may warm the water to undesirable temperatures and cause algal blooms which can cause larval mortalities.

Table 10. Survival of Japanese scallop embryos, *Patinopecten yessoensis*, reared at four salinities (Bourne and Hodgson, in prep.). For each experiment, the treatment with the 'A' Duncan grouping showed the highest survival. Treatments with the same Duncan grouping were not significantly different.

	Salinity (ppt)	Grouping
Experiment #1		
	29	A
	34	B
	25	B
	38	C
($r^2 = 0.97$)		
Experiment #2		
	29	A
	25	B
	34	B
	38	C
($r^2 = 0.97$)		
Experiment #3		
	29	A
	34	B
	25	C
	38	C
($r^2 = 0.99$)		

P>F	r^2	SOURCE	P>F
0.0001	0.98	SALINITY	0.0001
		TEMPERATURE	0.0001
		INTERACTION	0.0001

SALINITY	TEMPERATURE 10°C	15°C	18°C
25‰	0	3.9	0
29‰	45.9	72.9	79.3
34‰	10.4	21.7	0
38‰	0	0	0

P>F	r^2	SOURCE	P>F
0.0001	0.88	SALINITY	0.001
		TEMPERATURE	0.001
		INTERACTION	0.01

SALINITY	TEMPERATURE 15°C	18°C	21°C
25‰	100	100	0
29‰	80	93.3	53.3
31‰	58.3	68.3	0
34‰	0	0	0

Figure 27. Survival of Japanese scallop embryos, *Patinopecten yessoensis*, at three temperatures and four salinities (Bourne and Hodgson, in prep.).

5.5.1 Draining Larval Tanks

Water in larval tanks should be changed every 3-4 days. Tanks are drained through large siphons and the outflow is passed through a screen small enough to retain the larvae (Figure 29). Table 11 gives recommended screen sizes for collecting larvae of different sizes. The bottom of the collecting screen should be submerged in a tray of water to reduce the force by which the larvae are pressed against the screen. The larvae should not be left exposed to air on the screen when the tank stops draining. After the siphons have stopped draining, the bung at the edge of the tank bottom is popped and the remaining water is allowed to gently flow out of the tank. All larvae collected on the screen are resuspended in a known volume of water in a bucket or other container.

The tank is hosed down with FSW to collect any remaining larvae. These larvae are termed "dregs" and are put into a 4 l glass beaker. The dregs are left to sit for 15-20 minutes and any larvae that swim toward the top of the beaker are decanted into the bucket with the rest of the larvae. This method of collecting larvae from the dregs can be used if it is believed that some of them are healthy. If a large amount of bacteria and/or ciliates are present, it is best to discard the entire contents of the beaker.

The drained tanks are scrubbed with a dilute bleach solution (0.5%), rinsed with fresh water, rinsed with FSW and refilled with FSW before the larvae are resuspended.

5.5.2 Holding Larvae During Tank Changes

The length of time the larvae are held out of the tank should be kept to a minimum and should not exceed 2 hours. This is a stressful period for larvae and diseases that may be present could spread rapidly. Antibiotics can be added to the buckets to control the spread of diseases. A dual antibiotic treatment consisting of penicillin G (50 I.U./l) and streptomycin sulphate (50 mg/l) is used. On days when water in tanks is exchanged, a working solution of antibiotics is prepared; 1.8 gm Penicillin G (1670 I.U./gm) and 3.0 gm streptomycin sulphate dissolved in 60 ml distilled water. One ml of the working solution is added to every 1 l volume in the buckets.

Use of antibiotics in larval cultures is discussed further in the section on Antibiotics.

5.5.3 Counting and Measuring Larvae

Larvae are counted in the same manner as eggs. They are uniformly suspended in a known volume of FSW and a 1 ml sample taken. Since larvae are active swimmers, a few drops of 1% formalin are added to the sample on the slide to kill them and make counting easier. Shell lengths of 15 larvae are taken to the nearest 5 um using an ocular micrometer fitted into the eyepiece of a dissecting microscope. Instructions to calibrate an ocular micrometer are given in Appendix 2.

Table 11. Relationship between size of screen and size of larvae retained on it.

Screen Size (um)	Size of Larvae Retained (um)
44	D-stage (115 um) to 110
120	113 or larger
150	170 "
160	212 "
180	255 "
200	280 "

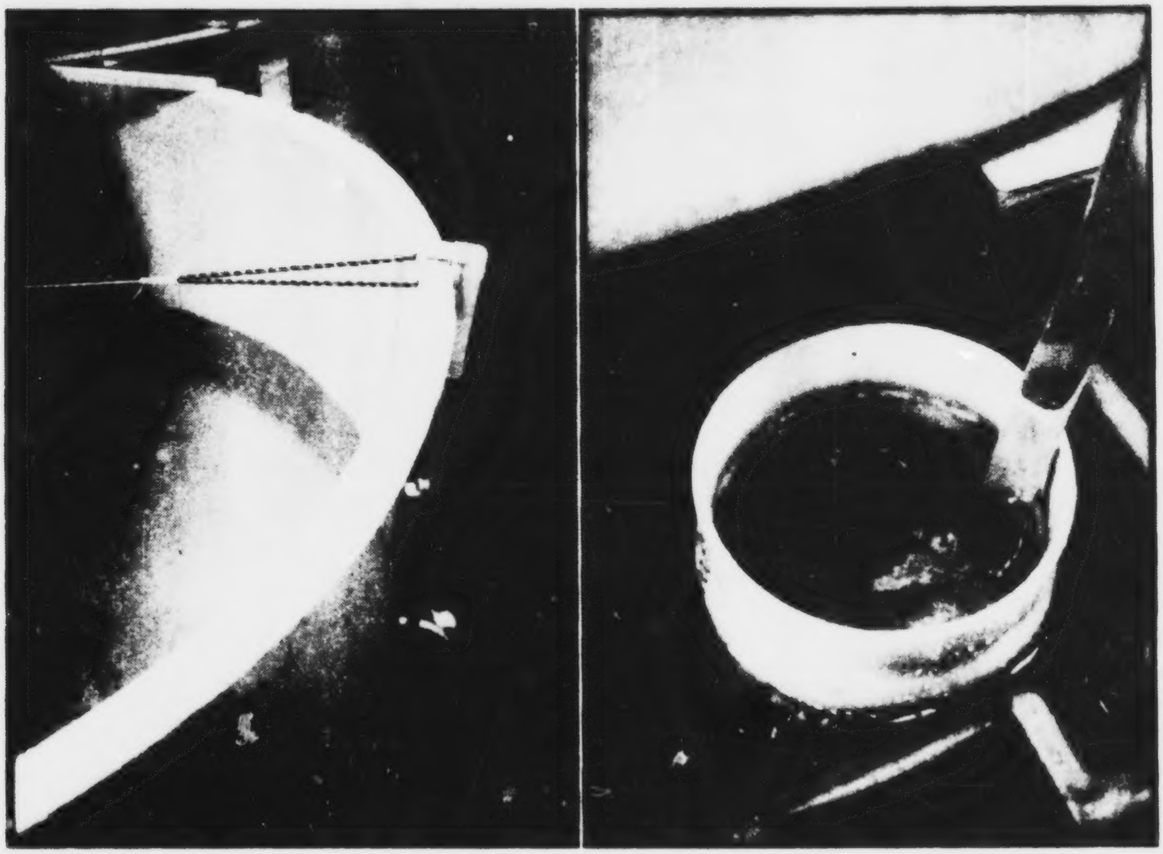

Figure 28. Survival of embryos of rock scallops, Crassadoma gigantea, at three temperatures and four salinities (Bourne and Hodgson, in prep.).

At the time of counting, larvae should be examined carefully to determine if they are healthy. Healthy larvae should have dark digestive glands, smooth shell margins and well-rounded and evenly ciliated velums.

5.5.4 Larval Density

It is important to ensure that larvae are not subjected to overcrowding in larval tanks because high levels of metabolic waste and physical damage caused by collision can occur. These conditions cause stress to the larvae and can lead to high mortalities. Recommended larval density varies with the age of the larvae. Early veliger larvae (3-10 days) can be held at densities of 1.5-1.75 larvae/ml, but older larvae (17 days to setting) should be held at densities no higher than 1 larva/ml.

5.5.5 Feeding

Once the straight-hinge larval stage is reached, the larvae are planktotrophic and feed on unicellular algae. The species and amount of algae fed depend on age of the larvae, density of larvae, and amount of algae consumed by the larvae. Table 12 summarizes the feeding protocol used at the Pacific Biological Station, which was developed after numerous feeding trials (Whyte et al. 1987, in press, Bourne and Hodgson, in prep.). Initially, *C. calcitrans* is fed to veliger larvae and the algal density in the larval tank should be 5,000 cells/ml. As the larvae grow, additional algal species are fed and the algal density is increased (Table 12). Consumption of algae is an indicator of the state of health of a larval culture, since healthy larvae will actively swim and graze the algae.

Algae are fed daily and the amount added depends on age of the larvae and the algal density remaining in the tank from previous feedings. The Coulter Counter is necessary for this feeding regime for it provides a measure of the amount of algae consumed by larvae. The Coulter Counter measures the number of particles in the seawater but does not discriminate between algal cells and other particles. In spite of fine filtration, there will be a low number of particles present in FSW. Before algae are added to the larval tank, an estimate of the particle count in the FSW is made and this is the background count. After the ration of algae is added to the tank, the total particle count of the tank will include both the background count and the algal count. Below is an example to determine algal density in a larval tank.

Particle count of FSW is 1,370 particles/ml
This is the Background count.
Larvae are 17 days old and therefore are fed
    5,000 cells/ml *T. pseudonana*
    5,000 cells/ml T. *Isochrysis*
    5,000 cells/ml *C. calcitrans* (Table 12)
Total = 15,000 cells/ml. This is the Algal count.
Therefore Target count for tank = Background (1,370) + Algal count (15,000) = 16,370 particles/ml.

Table 12. Algal feeding regime for scallop larvae of different ages.

Age (Days)	Algal Density in Tank (cells/ml)	Algal Species to Feed and Proportion
3-5	5,000	*Chaetoceros calcitrans*
6-10	10,000	2:1 *C. calcitrans* and *T*. *Isochrysis* sp.
11-15	12,000-15,000	1:1 *C*. *calcitrans* and *T*. *Isochrysis* sp.
16-21	15,000-20,000	1:1:1 *C*. *calcitrans*, T. *Isochrysis* sp., and *Thalassiosira pseudonana*
21-setting	20,000	1:1:2 *C*. *calcitrans*, T. *Isochrysis* sp., and *T*. pseudonana

On days when water in larval tanks is not changed, a sample of the water from each tank is collected and the particle count is calculated. If the count is below the desired target count, then more algae is added to bring the count up to the target level. Below is an example.

Present particle count in tank = 10,370/ml.
Target count for tank = 16,370/ml (from above example).
Difference = 6,000/ml.
Therefore, add algae to give total concentration of 16,370/ml in the tank.

The amount of algae added to the tank may seem low, but the objective is to maintain a constant algal density in the tank. Three factors affect the rate of algal depletion; grazing rate of larvae, larval density, and settlement of algal cells from the water. Some hatcheries add the same amount of algae to a tank each day. However, if the algae is not consumed at the rate at which it is added, there will be a build-up of algae in the tank (see next section).

5.5.6 Overfeeding

Overfeeding should be avoided. Not only is algae wasted, but high concentrations of algae in the larval tanks can be stressful to larvae. If the algal density in a tank becomes too high, then larvae stop feeding and larval growth and survival decline.

Larval tanks should be protected from bright light so that algal blooms do not occur and create an overfeeding situation since this will cause stress to the larvae.

5.5.7 Growth Rates

Growth rates of larvae are affected by diet, rearing temperature, and state of health. Figures 30-33 illustrate the differences in growth rates of Japanese and rock scallop larvae when reared at different temperatures, salinities, and fed different diets. Differences in salinity had little effect on larval growth (Figure 32). At the rearing temperatures used at the Pacific Biological Station (15°C for Japanese scallops and 18°C for rock scallops) and diets stated (Table 12), Japanese scallop larvae have an average growth rate of 6.9 um/day and rock scallop 4.8 um/day (Figure 34). Duration of the larval stage for the Japanese scallop is 21-28 days (15°C), at which time they are mature and have a shell length of 260-280 um (Figure 7). Rock scallop larvae mature when they are 220 um in length in approximately 20 days (18°C)(Figure 8).

Groups of larvae may grow slightly faster or slower, but should not be markedly different from average growth rates. Larvae growing much slower than average may indicate problems with the culture such as disease, improper feeding, or water quality.

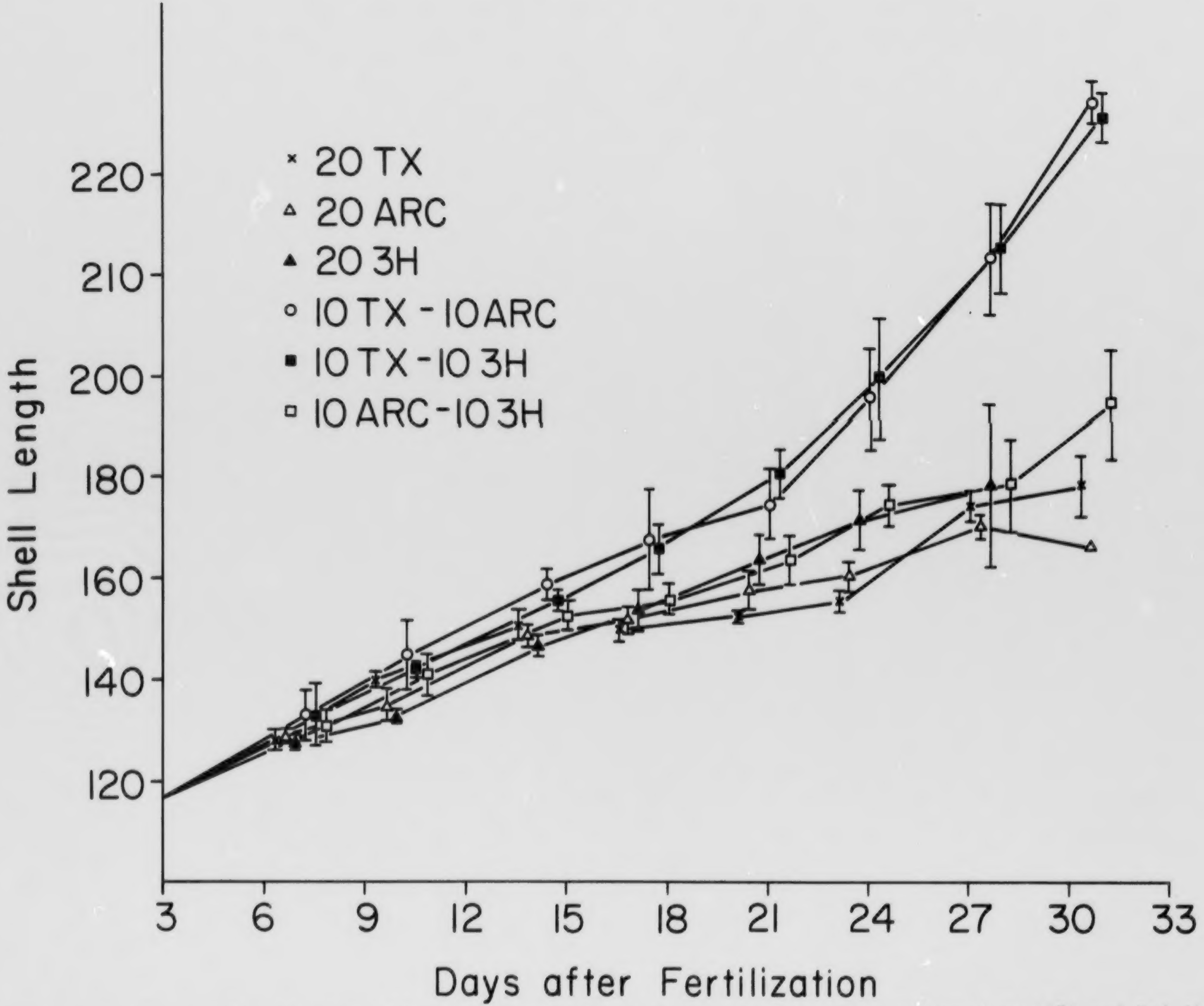

Figure 30. Growth of Japanese scallop larvae, *Patinopecten yessoensis*, when cultured at 15°C and fed different diets. TX, Tahitian *Isochrysis*, ARC *Chaetoceros calcitrans*, 3H, *Thalassiosira psuedonana*, (Bourne and Hodgson, in prep.).

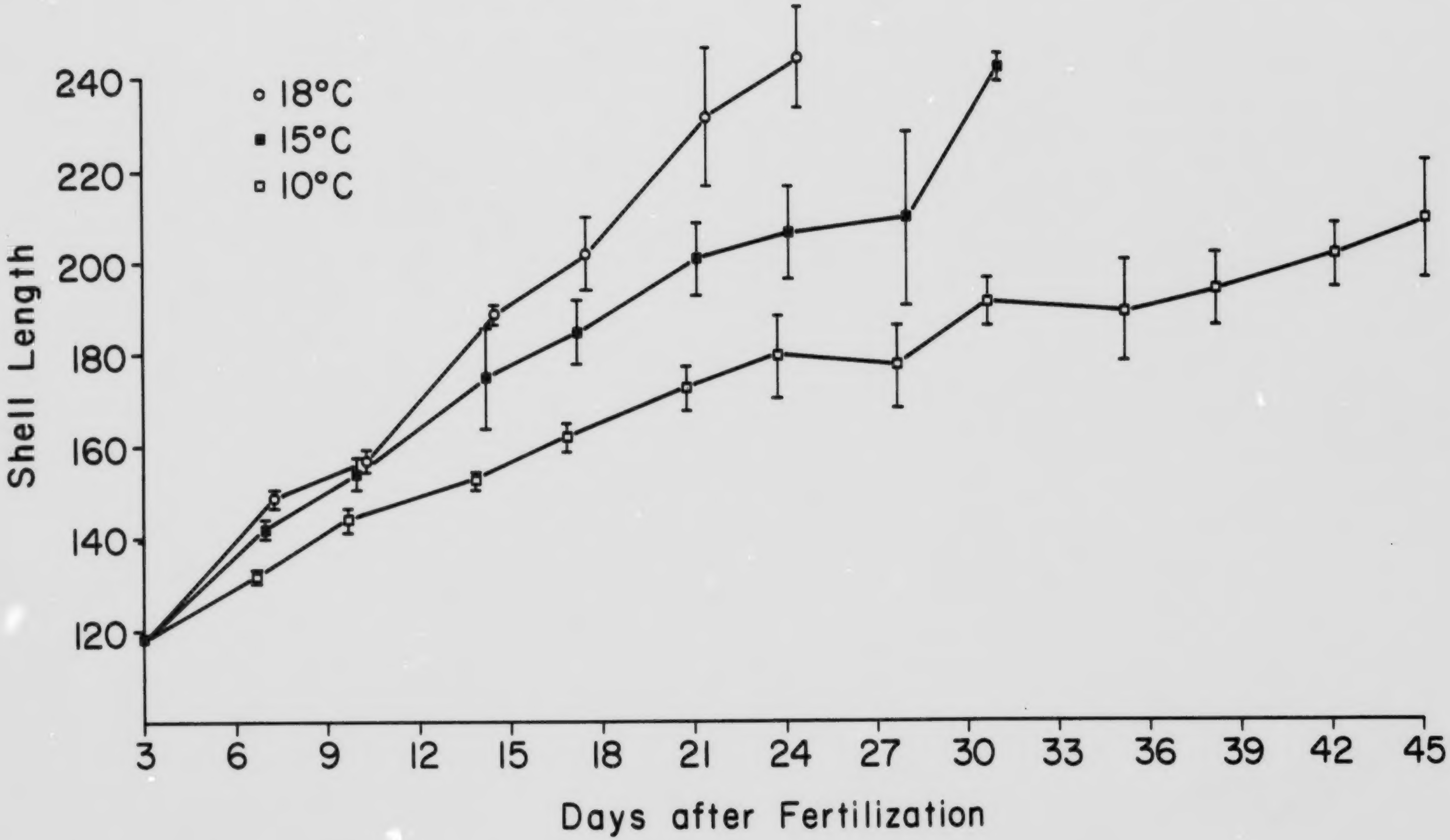

Figure 31. Growth of Japanese scallop larvae, Patinopecten yessoensis, at three temperatures at 29 ppt salinity (Bourne and Hodgson, in prep.).

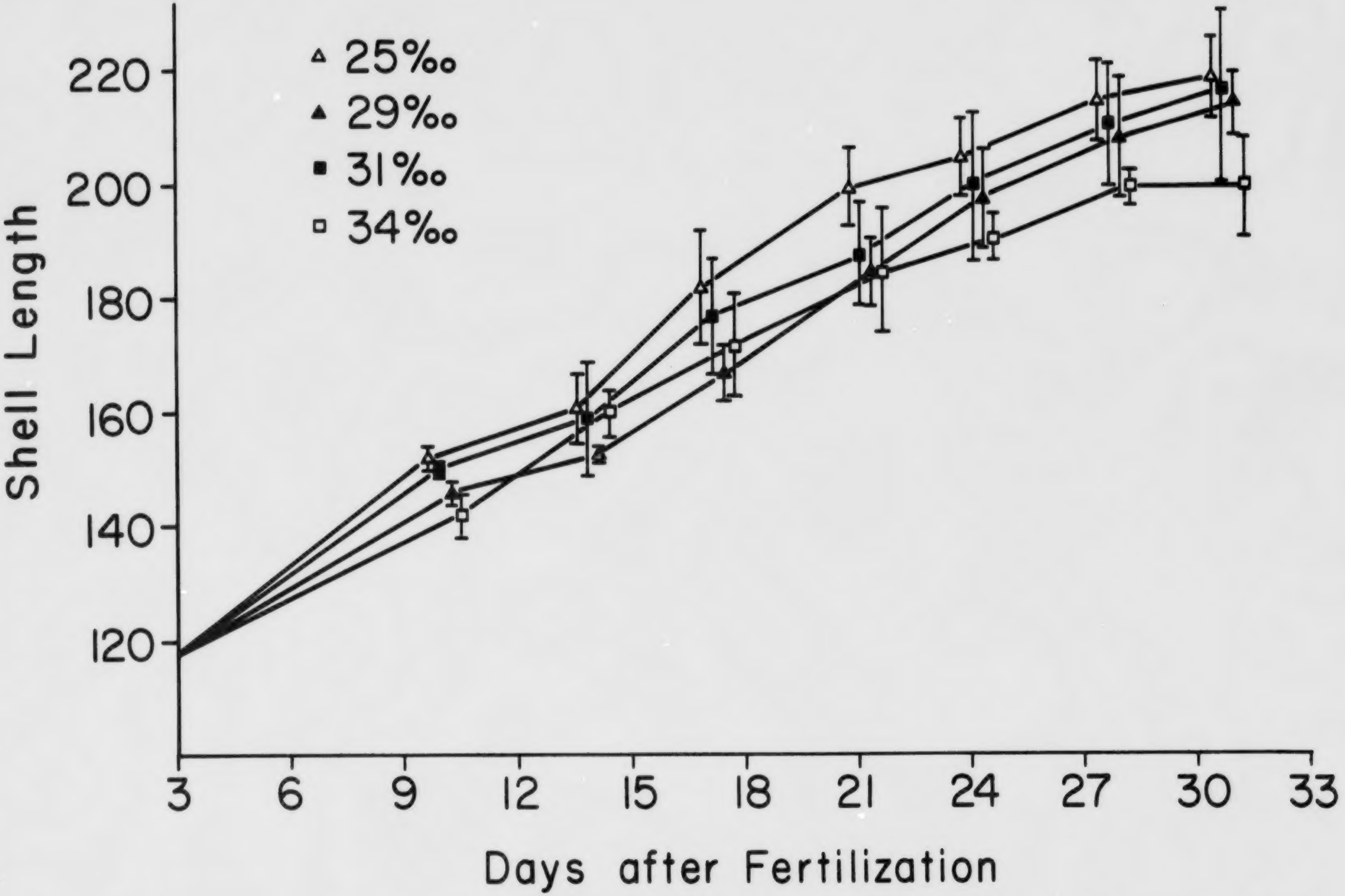

Figure 32. Growth of Japanese scallop larvae, Patinopecten yessoensis, raised at four salinities at 15°C (Bourne and Hodgson in prep.).

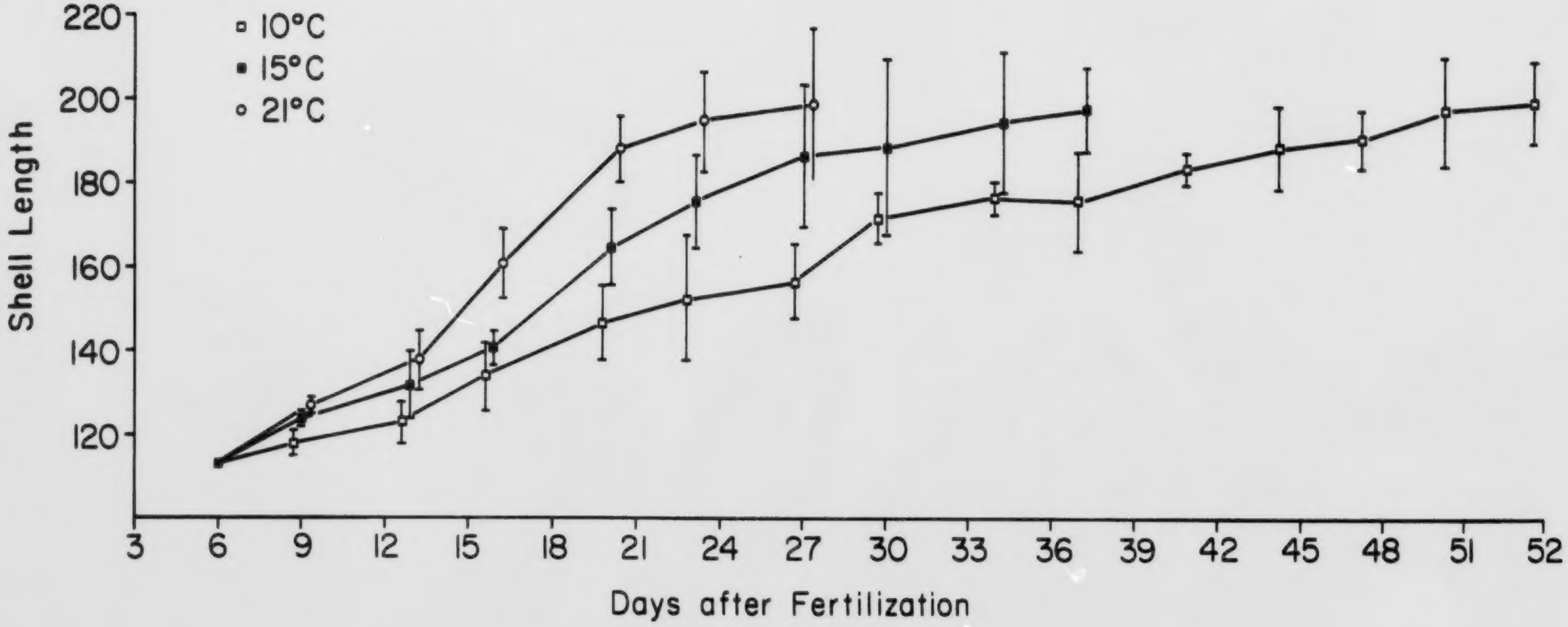

Figure 33. Growth of rock scallop larvae, *Crassadoma gigantea*, at three temperatures at 29 ppt salinity (Bourne and Hodgson in prep.).

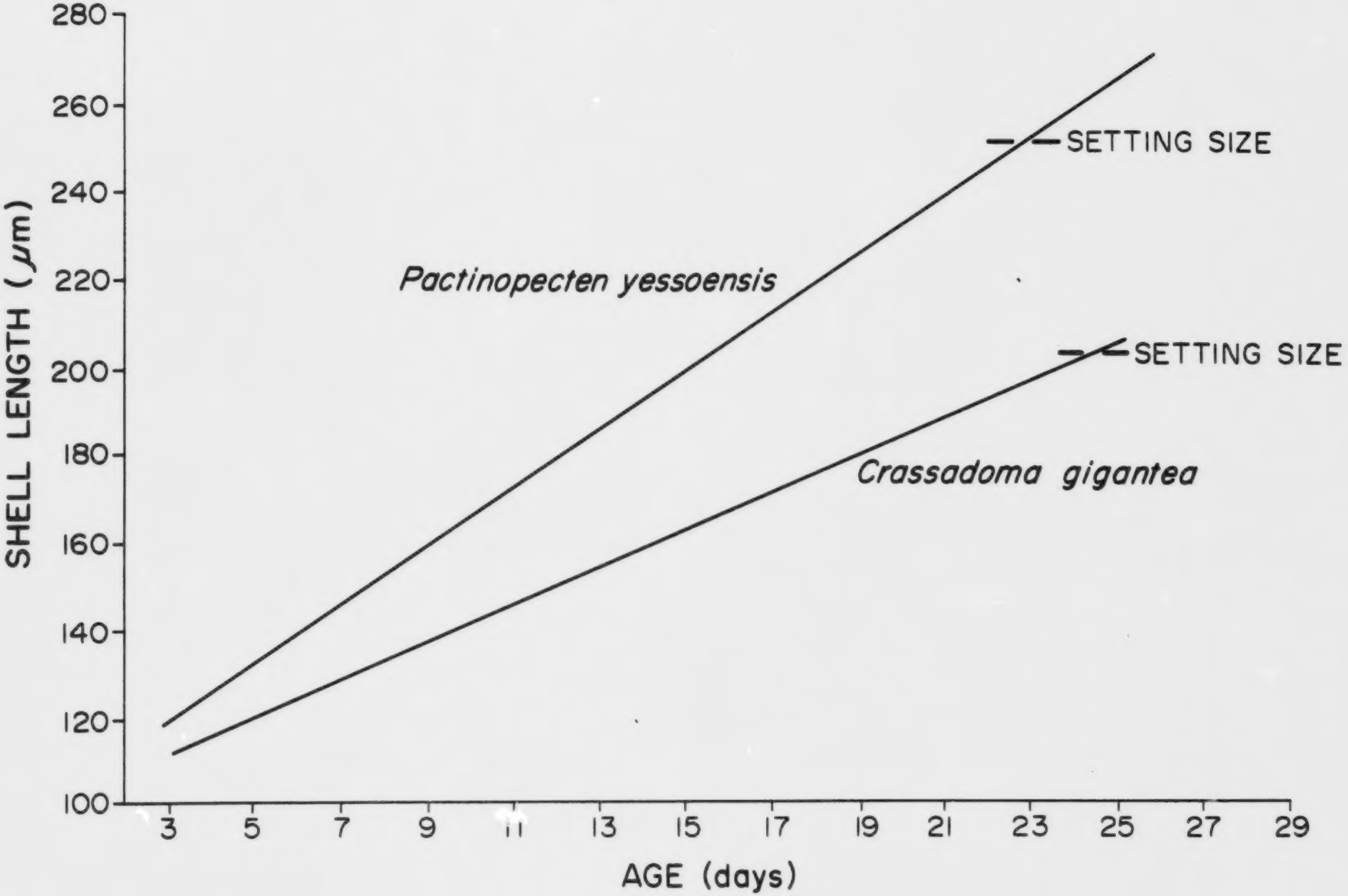

Figure 34. Average growth curves of Japanese and rock scallop larvae reared at the Pacific Biological Station.

5.5.8 Screening Larvae

As a group of larvae gets older, the size range of larvae within the group becomes larger. Initially, all larvae are approximately 120 +/- 5 um, but after 2 weeks there may be as much as a 25 um size spread between the largest and smallest larvae. Some commercial hatchery managers believe that growth of the smaller larvae is inhibited by larger ones. Therefore, larvae should be sorted routinely into homogeneous size groups.

Larvae are first sorted about 14 days after fertilization. Generally, larvae should be sorted when the size range of a group exceeds 25 um. Larvae are sorted into size classes by passing them through a series of different mesh screens. The smallest mesh size should be the same as used to collect the larvae when the tank was drained. Stacked above this screen are screens of increasing mesh size so that the largest mesh size screen is on top. Larvae are poured through the stack and gently rinsed with FSW (Figure 35). The more screens used, the more size classes of larvae are sorted. Usually, larvae are sorted into 2-4 size classes. Larvae from each size class are resuspended in FSW, counted, measured, and placed into appropriate tanks. It is important to ensure the larvae are left on the screens for a minimum amount of time and are constantly rinsed with FSW.

5.5.9 Poor Spawns

On occasion, a group of larvae from a spawn may have poor growth, although they are held under correct culture conditions. Eggs at the time of spawning may not have been fully ripe or had low energy reserves, and the percent fertilization of these eggs is much lower than expected. Poor fertilization rate generally coincides with poor growth and survival of the resulting larvae. A few larvae from a poor spawn may survive to metamorphosis. Once a poor spawn has been recognized, the larvae should be discarded since it is not worthwhile to raise so few larvae.

5.6 Disease

Periodically, groups of larvae will die suddenly and the entire culture is lost. High bacterial counts are almost always associated with large larval mortalities. It is uncertain whether the bacteria cause the mortalities (pathogenic) or are simply present as opportunistic bacteria (saprophytic) to feed on dying larvae. Bivalve larval diseases are known to occur and every precaution must be taken to prevent epidemics developing in a hatchery (Bolinches et al. 1986, Elston et al. 1987).

To control disease outbreaks in a hatchery, larvae should be inspected regularly. Unhealthy larvae often have misshapen velums, poor colour, and slow growth rates. Other indicators of a possible disease are reduced algal consumption rates and appearance of brown spots or patches on the bottom of larval tanks. Larvae and the patches should be examined carefully.

Figure 35. Screening larvae. Larvae are rinsed through a series of screens stacked in descending mesh size.

A patch can be taken out of a tank by using a long piece of glass tubing or at the time of water exchange. Hold one end of the tubing closed with a finger and position the other end over one of the patches. Release the finger and the patch will be sucked up the tubing. Close off the end of the tubing again to remove the sample from the tank.

The patches generally are groups of larvae stuck together in a glutinous mass with numerous protozoa and bacteria. On occasion, a fungal infection may be present and can be identified by numerous thread-like projections in the mass or in the larval tissues.

If a disease is suspected in a tank, the tank should be drained immediately and thoroughly scrubbed with a 1% bleach solution. Depending on the severity of the disease, a decision must be made whether it is advisable to discard all the larvae or to separate and discard only the moribund larvae. Larvae from the bottom of the tank (dregs) should be discarded regardless. The remaining larvae from the diseased tank can be isolated, rinsed with large amounts of FSW, and treated with antibiotics (see section Holding Larvae During Tank Changes).

To protect larvae from further contamination, the larval tank can be refilled with UV-irradiated FSW. UV-irradiation sterilizes the seawater and kills bacteria. Larvae themselves cannot be exposed to UV-light as it will kill them. Details on the function of a UV-sterilizer are provided in Appendix 3.

All materials used for handling the diseased larvae must be chlorinated and rinsed to prevent the spread of the disease.

5.7 Cleanliness in a Hatchery

Proper hygiene in the hatchery cannot be over-stressed. Continuous vigilance is necessary to prevent disease outbreaks and control their spread. Many bacterial problems are due to poor water filtration systems or lack of cleanliness (Lewis et al 1986).

All equipment must be washed and dried routinely. After use, an item should be washed immediately, rinsed and allowed to dry. Hoses should be rinsed daily with fresh water and dried. Periodically, all hoses and pumps should be chlorinated and rinsed. Larval screens should be dipped in a bleach solution and rinsed after each use. Buckets used to hold larvae should be clearly marked and not used for other purposes. Similarly, buckets and brushes used for scrubbing tanks should be clearly marked and used for that purpose only. All siphons should be stored soaking in a bleach solution or rinsed and hung to dry.

Filters in the seawater lines should be removed, rinsed in fresh water and dipped in a bleach solution when FSW is not required. If the filters are not removed, a slow, constant flow of water should pass through them to prevent stagnation of the seawater in the filters.

Periodically, all equipment should be rinsed in bleach or washed with biodegradable soap.

5.8 Antibiotics

Experimental work at the Pacific Biological Station has shown that use of antibiotics can improve growth and survival of larvae (Figure 36). However, routine use of antibiotics in a commercial bivalve hatchery should be avoided because the cost of antibiotics is prohibitive and there is the possibility of producing resistant strains of bacteria. Occasional use of antibiotics may be used to treat specific problems.

Antibiotics can be administered in two ways. Larvae can be held in an antibiotic bath solution for a short period of time, such as when larvae held in 10 l buckets inoculated with antibiotics during water changes. An alternative method is to add antibiotics directly to the larval tanks. The advantage of a short term treatment is that small quantities of antibiotics are required but it may not be as effective in controlling diseases as when antibiotics are added to larval tanks.

At the Pacific Biological Station, a mixture of two antibiotics consisting of streptomycin sulphate and penicillin G is used. A working solution of 3.0 gm streptomycin sulphate and 1.8 gm penicillin G is dissolved in 60 ml distilled water and is prepared on the day it is required. One ml of the working solution is added to every 1 l volume of FSW in which the larvae are held.

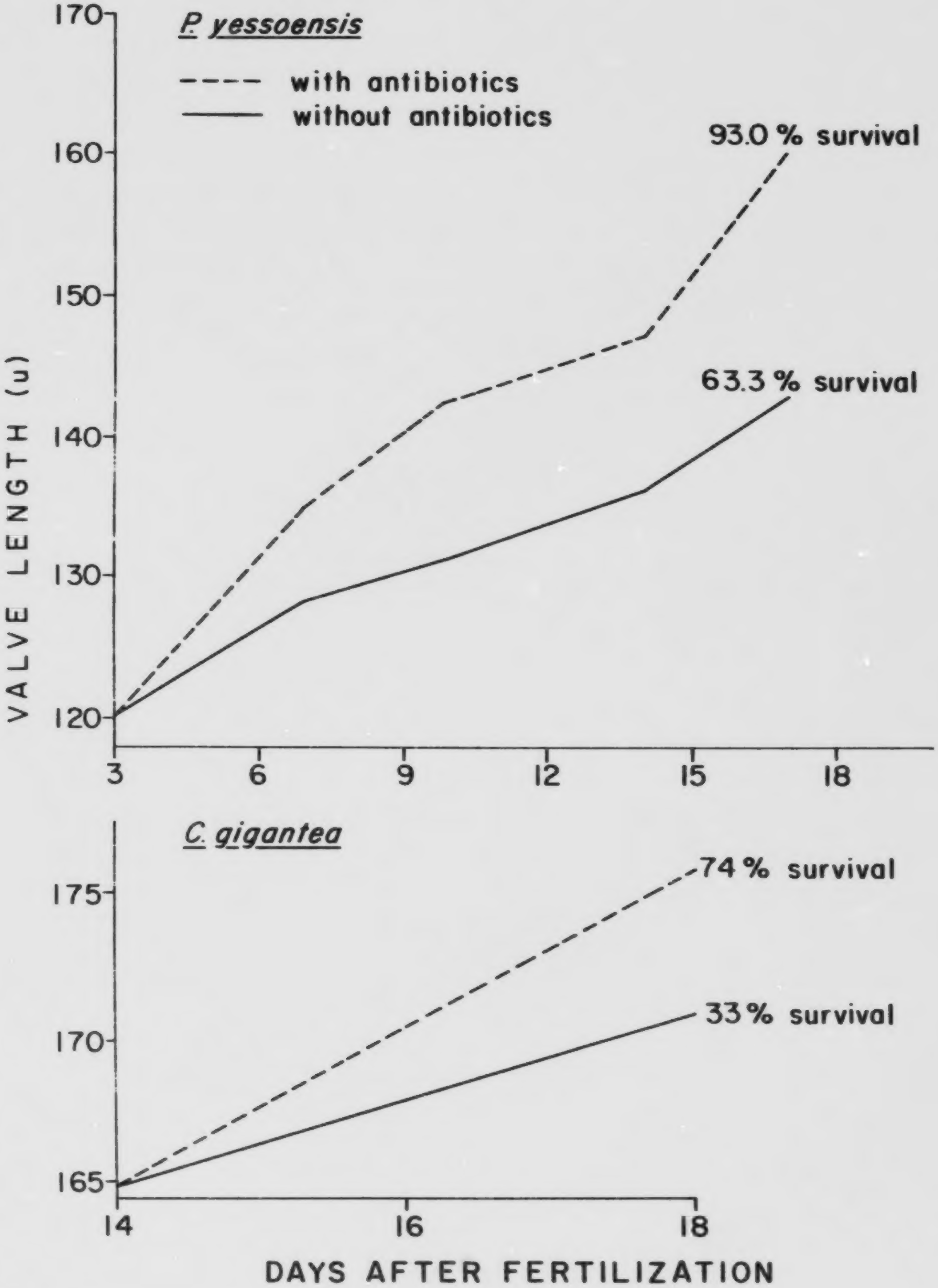

Figure 36. Growth and survival of Japanese scallop larvae, Patinopecten yessoensis, and rock scallop larvae, Crassadoma gigantea, cultured with and without antibiotics (Bourne and Hodgson, in prep).

6. NURSERY

The goal of the nursery is to grow large quantities of juvenile scallops quickly from setting size (260 um) to approximately 40-50 mm shell height, at which time they can be outplanted and grown to commercial size (10 cm shell height).

The nursery can be divided into two stages. The first stage, referred to here as the primary nursery, encompasses growth of juveniles from settlement to about 10 mm shell height. The second stage, the secondary nursery, encompasses growth of juveniles from 10 mm shell height to 40-50 mm, and corresponds with the intermediate phase of scallop culture in Japan (Ventilla 1982).

6.1 Primary Nursery

The primary nursery can be further divided into two phases: phase 1, growth from settlement to 1 mm shell height, and phase 2, growth to 10 mm shell height.

6.1.1 Phase 1: Setting to 1 mm Shell Height

At the completion of the larval stage, the larvae are mature and capable of metamorphosing into juveniles. Mature larvae are identified, separated from others and placed in a setting system. Metamorphosis and the period immediately after is a critical stage in the life of scallops and high mortalities can occur at this time. Early juveniles are extremely fragile and highest mortalities appear to occur when they measure 0.4-0.6 mm shell height. Cause of the mortality is unknown but may be due to poor nutrition or improper handling.

6.1.2 Identification of Mature Larvae

Criteria used to determine mature larvae include size, morphology and behaviour. Mature Japanese scallop larvae are 260-280 um in shell length and mature rock scallop larvae are 220 um in length (Figures 7 and 8). A well-developed foot, developing gill bars, and eyespots approximately 10 um in diameter aid in the identification mature larvae. At maturity, the behaviour of the larvae changes from continuous swimming to periods of swimming interspersed with periods of crawling with the foot on a substrate. Foot extension while swimming is also indicative of mature larvae.

6.1.3 Screening Mature Larvae

When mature larvae are observed during routine inspection, the larval culture should be screened to separate them from the rest of the larvae. Mature larvae are collected on a 180 or 200 um screen, following the procedure outlined in the section Screening Larvae (5.5.8). A larger screen, such as a 250 um mesh size, can be placed on top of the stack of screens to collect any debris that may be in the culture. Mature larvae are rinsed from the 180 or 200 um screen, resuspended in a bucket of FSW (15°C), counted and measured before placing them into a setting system.

Mature larvae often will clump together to form mucous strands in the water (Figure 37). This behaviour is called "rafting" and is a sign of healthy and vigorous larvae that are ready to metamorphose.

6.1.4 Setting Systems

Mature larvae are placed into setting systems to undergo settlement, metamorphosis and development into juveniles. Setting systems have particular features, such as cultch, bacterial films, and water flow, which are believed to stimulate metamorphosis.

6.1.4.1 Cultch

Prior to metamorphosis, scallop larvae stop swimming and attach themselves to a substrate. Artificial substrate, called cultch, greatly increase the amount of surface area in a setting system. If no material is provided, larvae can only settle on sides and bottom of tanks.

As with most molluscan species, scallop larvae are believed to be selective about the type of substrate to which they attach before beginning metamorphosis (Meadows and Campbell 1972, Crisp 1974, Burke 1983). The behaviour of periods of swimming interspersed with periods of crawling is believed to allow them to test different substrate. If a suitable substrate is not located, larvae can delay metamorphosis and may actually die as larvae (Bayne 1965, Crisp 1974, Sastry 1979, Hodgson and Bourne, 1988).

Several types of material have been tested as cultch for scallop larvae: bark, palm leaves, monofilament, scallop shell, pebbles, unlaid polypropylene line (Culliney 1974, Motoda 1977). Materials that have been tested at the Pacific Biological Station include oyster and scallop shell, monofilament, polypropylene line, vexar, artificial turf, jute, sisal, and kinran. Of these, the best material is kinran, an artificial fibre manufactured in Japan (Figure 38).

6.1.4.2 Bacterial Film

A film of bacteria or micro-organisms on a surface is known to influence settlement and metamorphosis of some marine invertebrate larvae, including bivalves (Cole and Knight-Jones 1949, Walne 1974, Hodgson and Bourne 1988). Scallop larvae may be affected by microbial films on the cultch (Hodgson and Bourne 1988). Cultch used for setting should be soaked in FSW

Figure 37. "Rafting" of mature larvae. Larvae clump together in mucous strands when concentrated in a small volume of water.

Figure 38. Artificial fibrous cultch material, kinran, in setting tank.

for a minimum of 10 days prior to the addition of mature larvae to encourage growth of a potentially beneficial microbial film.

6.1.4.3 Water Flow

Water movement may also increase the rate of settlement and metamorphosis (Crisp and Meadows 1962, Hodgson and Bourne 1988). A water circulation system can be an inherent part of the setting system.

6.1.5 Chemical Induction of Settlement and Metamorphosis

Introduction of chemicals such as L-Dopamine, GABA, potassium chloride, norepinephrine, epinephrine, and acetyl-a-choline have been shown to induce settlement and metamorphosis in molluscan larvae (Morse, et al 1979, Coon and Bonar 1985, Coon et al. 1986). GABA, L-Dopamine, epinephrine, and potassium chloride were tested at the Pacific Biological Station but did not induce Japanese or rock scallop larvae to settle and metamorphose.

At present, chemicals are not used to induce settlement and metamorphosis in scallop larvae at the Pacific Biological Station.

6.1.6 Thermal Stimulation

A sudden decrease in water temperature may influence the onset of settlement (T.M. Wong, unpubl. MS). The rate of settlement and metamorphosis for rock scallop larvae increased when larvae were chilled to 5°C for at least 30 minutes. Larvae were held in a water bath (15°C) and gradually cooled over a 20 minute period to 5°C. Larvae were held at 5°C for 30, 60, or 90 minutes and then allowed to return to room temperature. The proportion of larvae that metamorphosed when held at the cool temperature was significantly higher than larvae held at the control temperature of 15°C, but did not differ with length of the cooling time. In a second experiment, rock scallop larvae were cooled to 8°C with similar results.

At the Pacific Biological Station, mature scallop larvae are routinely cooled from 15°C to 8°C in a 10 l bucket before they are placed in a setting system.

6.1.7 Setting Tank

Several types of setting systems have been tested at the Pacific Biological Station. The most successful method has been to set the larvae in a large tank (2500 l) filled with FSW and kinran. The tank is gently aerated to maintain dissolved oxygen levels and to circulate the water. This system requires little maintenance.

6.1.7.1 Setting Density

The number of larvae placed in a setting system is critical. If too many larvae are put into the system, the rate of settlement, metamorphosis and subsequent post-larval survival declines. Initial studies at the Pacific Biological Station showed that setting density should be no higher than 2 larvae/ml (Table 13). More recent research has shown that setting density should be no higher than 0.5 larva/ml.

6.1.7.2 Feeding

Setting larvae are fed in the same manner as other larvae (Section 5.5.5). A mixed diet of T. *Isochrysis*, *C. calcitrans*, and *T. pseudonana* is recommended at levels of 20,000-25,000 cells/ml.

Observations suggest scallop spat may use an alternate type of feeding between the time the velum is lost and the gill filaments are able to take over the feeding process. The gill filaments may take 2-3 weeks after metamorphosis to develop into an efficient filtering apparatus. During this time, spat may not be able to filter-feed and instead may feed on particulate material adhering to surfaces. The foot may be used for this feeding process.

Experimental work at the Pacific Biological Station supports this hypothesis. Soon after setting, the particle count in a setting tank declines only slightly, indicating that little of the supplied phytoplankton is filtered from the water. This may continue for up to 3 weeks and then suddenly, the particle count will decline significantly. This decline may indicate that the juveniles have begun to actively filter food from the water.

6.1.7.3 Changing Water in Setting Tanks

FSW in setting tanks is changed 2-3 times weekly. Setting tanks cannot be drained, scrubbed, and refilled as larval tanks since spat will detach from the cultch if they are exposed to air. Water in a setting tank is exchanged by adding FSW at the same rate that water is siphoned from the tank so that the water level does not change as water is exchanged. Water siphoned from the tank should be passed through a 180 um screen to retain larvae that may be siphoned from the tank. For an effective water exchange, approximately twice as much FSW should be added to the tank as the actual volume of the tank. If a tank has been properly exchanged, the background count of the tank should be less than 5,000 particles/ml.

6.1.8 Duration of Setting

The length of time required for larvae to settle and metamorphose depends on maturity and health of the larvae, and the presence of stimuli. The actual process of metamorphosis usually is complete within 48 hours (Hodgson and Burke 1988) but this may be delayed because of unsuitable conditions in the setting system.

Table 13. Patinopecten yessoensis spat when set at different densities (Bourne and Hodgson, in prep.).

Density (larvae/ml)	Date set	Mean setting	% Metamorphosis size (um)
0.5	July 25	258.9	60, 80
1.0	Aug. 1	258.7	90, 90
2.0	July 28	268.7	72.5, 90
4.0	July 25	258.7	35, 30
8.0	July 28	268.7	62.5, 70.6
14.0	Aug. 1	258.7	33, 35

If larvae are still observed swimming in the setting tank three weeks after setting, probably they were not in good condition or the conditions in the setting tank were not suitable. In either case, success through metamorphosis for such a group of larvae probably will be low and it will not be worthwhile to maintain them longer in the nursery phase.

Once the larvae have metamorphosed, they are spat (juveniles) and are approximately 270-300 um in shell height. Spat are held in the setting tanks, and handled in the manner as described above for setting, until they reach a size of 1 mm shell height. Generally, the time to reach this size is 3-4 weeks.

6.2 Phase 2: 1 mm to 10 mm Shell Height

Five types of culture systems have been examined to rear juvenile scallops from 1 mm to 10 mm shell height: recirculating tank, flow-through tank supplied with surface seawater, downwellers and upwellers, raceways, and offshore nursery.

6.2.1 Recirculating Tank

A recirculating tank is similar to a setting tank but is equipped with a recirculating pump to increase the rate of water movement (Figure 39). An inner-outer standpipe arrangement allows water to be drawn from near the tank bottom and to be pumped in a fan pattern near the water surface, causing water circulation from the bottom to the top of the tank in a circular pattern. A nitex screen (250 um) is placed over the openings of the outer standpipe to prevent entrainment of any unattached spat. Water exchanges are undertaken twice weekly.

6.2.2 Nursery Tanks Supplied With Surface Seawater

The seawater supply for the hatchery is filtered and has little natural phytoplankton. To avoid having to raise large quantities of food in the algal culture facility, natural phytoplankton from the surface waters can be used. Nursery tanks are supplied with a constant flow of seawater drawn from about 3 m below the surface of the ocean and filtered to 50 um (Figure 40). The intake line to the nursery tanks should be as short as possible to reduce work required to clean the lines from fouling.

In Departure Bay, coulter counter counts of the surface seawater generally are about 15,000-20,000 particles/ml. In experimental work, this was augmented by periodic additions of cultured phytoplankton to bring the total particle count up to 20,000-35,000/ml. Results indicated that up to four times as many juvenile scallops were recovered from this system as compared to juveniles recovered from a recirculating system.

A difficulty with this system may be encountered in maintaining correct water temperatures. In Departure Bay, surface water temperatures

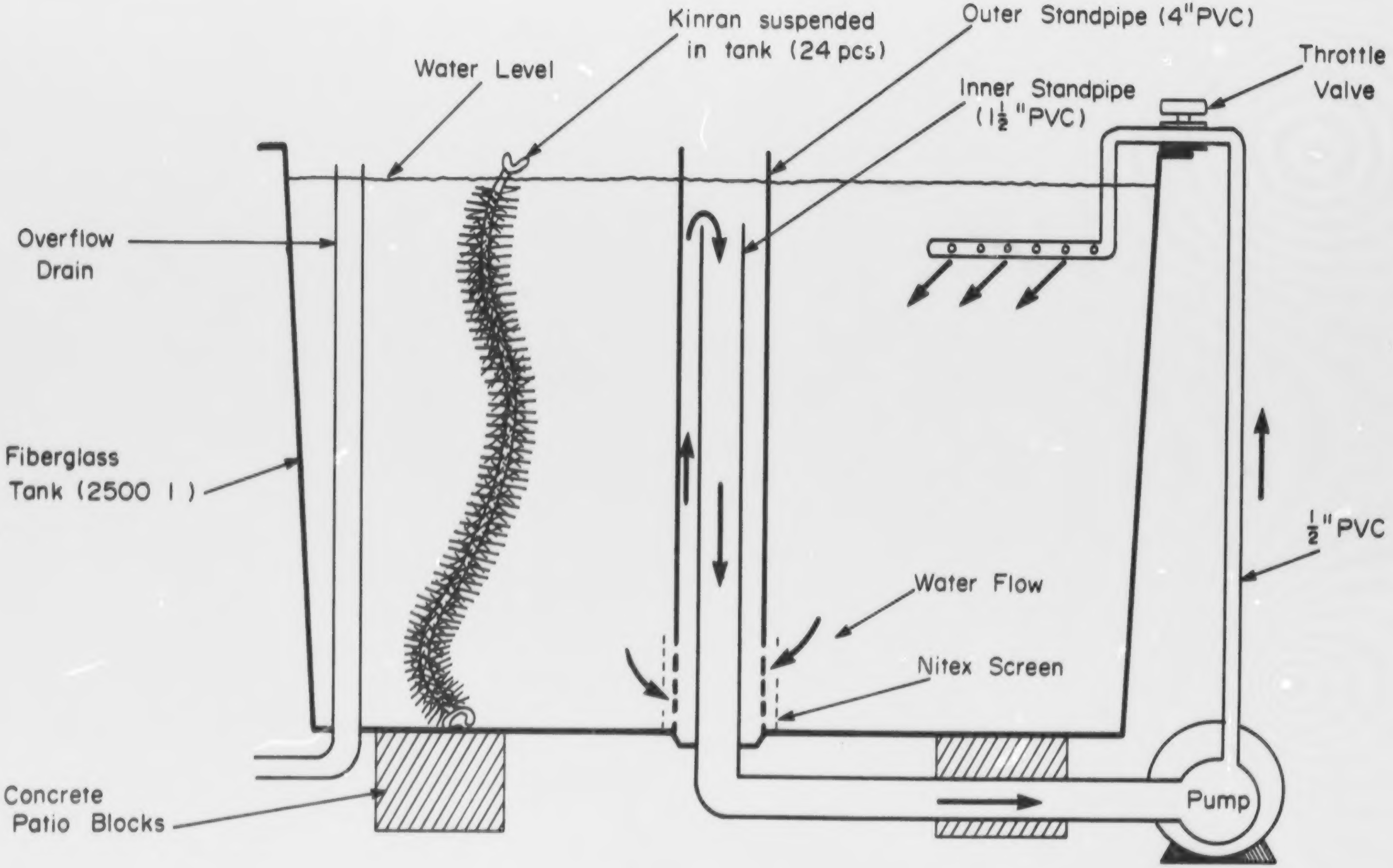
Kinran suspended in tank (24 pcs)
Outer Standpipe (4"PVC)
Water Level
Throttle Valve
Inner Standpipe (1½"PVC)
Overflow Drain
Fiberglass Tank (2500 l)
½"PVC
Water Flow
Nitex Screen
Concrete Patio Blocks
Pump

Figure 39. Diagram of a recirculating nursery system.

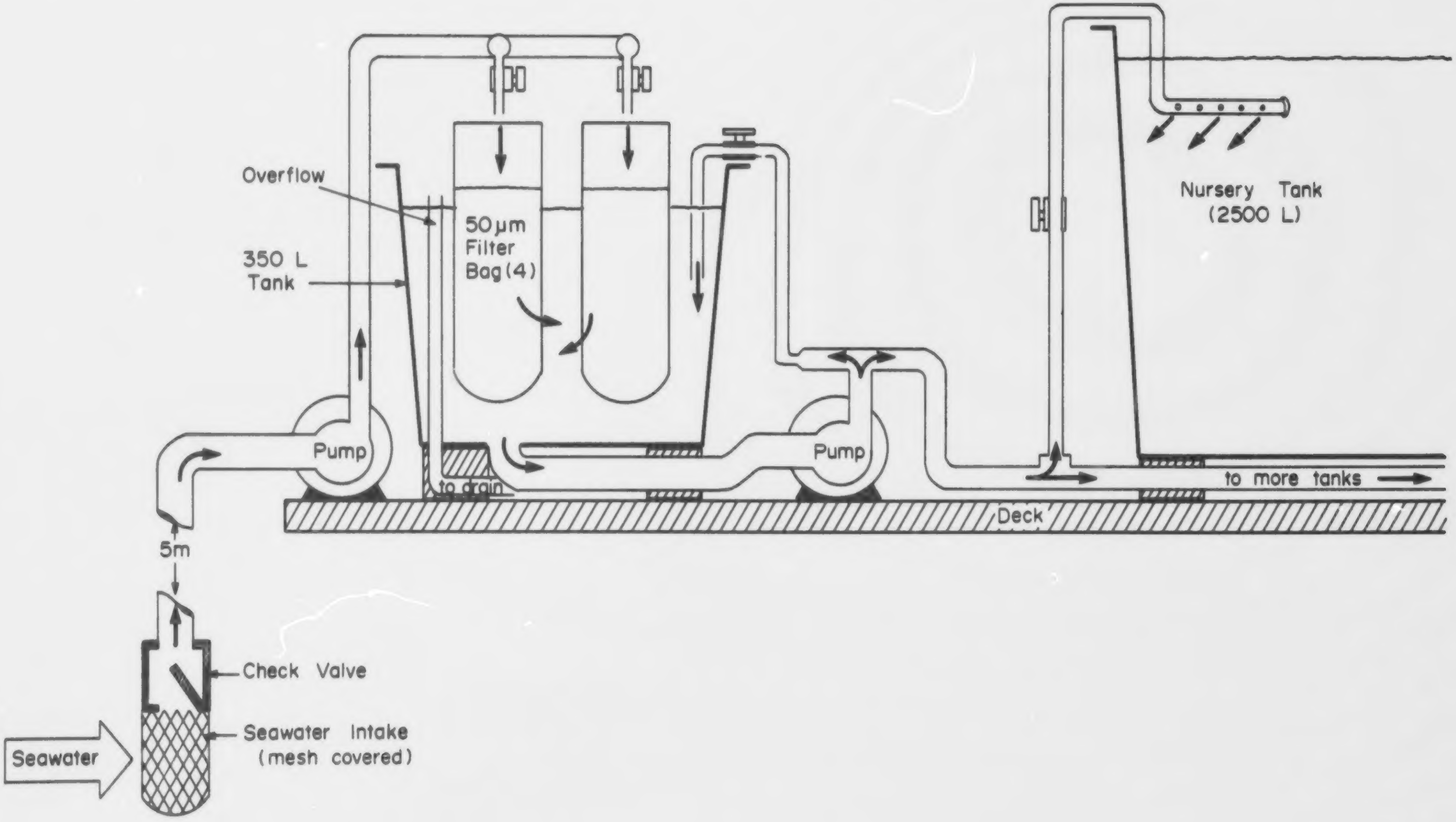
Overflow
350 L Tank
50 µm Filter Bag (4)
Pump
Pump
to drain
Nursery Tank (2500 L)
to more tanks
Deck
5m
Check Valve
Seawater Intake (mesh covered)
Seawater

Figure 40. Diagram of a nursery system supplied with surface sea water.

increase to 20°C in summer months, which is an unacceptable temperature for juveniles.

6.2.3 Downwellers and Upwellers

Upwelling and downwelling systems are used as nursery systems in commercial oyster and clam hatcheries. These are basically containers with a mesh screen attached to the bottom that is small enough to retain the spat. An inlet/outlet is placed at the side of the container to allow upward or downward water circulation. In downwellers, the water is passed into the container from above and then down through the bottom screen. In an upwelling system, water is moved up through the bottom screen and then out an opening on the upper side of the container. With upwelling systems, a screen should be placed over the outlet to avoid loss of any spat.

Upwellers/downwellers can be made into recirculating systems by placing them in a large tank. A large volume of water can be held in the tank to serve as a reservoir and is recirculated through the upwellers/ downwellers with a pump. The water in the system is exchanged with FSW at least twice weekly. Upwellers/downwellers can also be used in a flow-through system supplied with surface seawater filtered to 50 um. Flow rates vary with the shape and size of the container and should be between 10 l/minute and 20 l/minute.

6.2.4 Raceways

A raceway is a shallow trough with an inflow at one end and an outflow at the other. Water passes over the spat in a laminar flow. Raceways usually are set up as a continuous flow-through system, but can be modified to be a recirculating system. A disadvantage is that they require a large amount of space.

6.2.5 Offshore Nursery

In an off-shore nursery, spat are held in the open environment. When spat are 1 mm shell height, they are firmly attached to the cultch and can be placed in spat bags or onion bags and suspended in open environment (Figure 10). Mesh size of spat bags is 1-4 mm and this is important for maximum growth and survival of spat (Figure 41). If the mesh size is too small, there will not be an adequate water exchange through the bags. If the mesh is too large, spat that detach from the cultch will not be retained. When transferring spat to the offshore nursery, the time that spat are removed from the water must be kept to a minimum.

Spat bags are placed in the open ocean so that juveniles can feed on natural phytoplankton. This method allows juveniles to feed on a wider range of phytoplankton, which is nutritionally advantageous. Spat bags are suspended from a long-line or float and are held at a depth of 5 to 10 m. The depth is site specific. It is important to suspend them at a depth where they

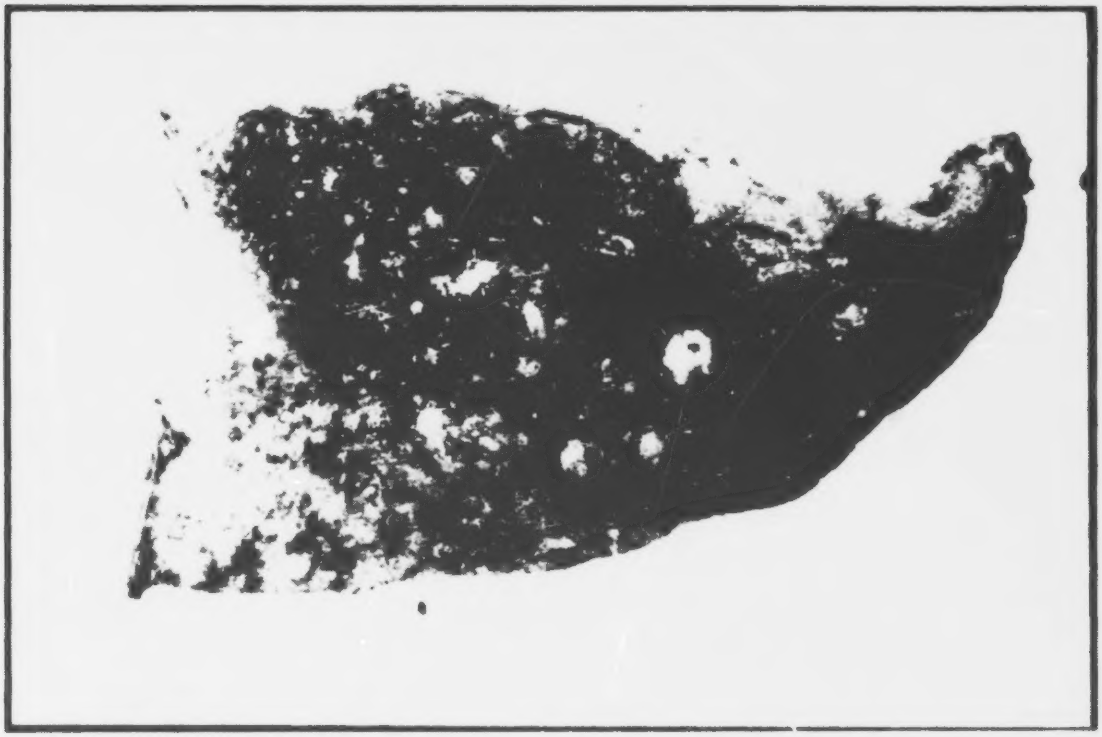

Figure 41. Spat bag containing kinran and attached scallop spat.

will be unaffected by high temperatures and low salinities, but not so deep that the spat cannot benefit from the phytoplankton in the seawater.

A critical stage in the offshore nursery is when to transfer spat from the setting tank to the offshore nursery. Until 1987 at the Pacific Biological Station, spat were held in recirculating tanks for 10-12 weeks, at which time they were approximately 5 mm shell height, and then transferred to the offshore nursery. The possibility of transferring 3 week old spat (0.5-0.7 mm shell height) from recirculating tanks to the offshore nursery was examined. More spat were recovered when held in this manner than when held in recirculating tanks until 10 weeks after metamorphosis. These results indicate that the offshore nursery system is more efficient for holding spat than the other nursery systems. Current practice at the Pacific Biological Station is to hold spat in recirculating tanks for 3-5 weeks and then transfer them to the offshore nursery. This assures that spat are firmly attached to the cultch before they are transferred to the offshore nursery.

6.2.6 Feeding

In a recirculating system, food levels are monitored in the same manner as larval and setting tanks (Section 5.5.5). Food levels should be maintained at 30,000-35,000 cells/ml and all phytoplankton species listed in Table 3 can be used as food. If food levels begin to drop markedly in 24 hours, feeding should be increased to 40,000-50,000 cells/ml and tanks should be checked twice daily.

A major food component for juveniles in a flow-through system is natural phytoplankton in seawater, but this can be augmented with cultured algae. A continuous drip feed system should be installed so that food levels remain relatively constant. This requires calculation of the cell concentration of the algae to be added and the flow rate through the system, so that food levels in the tank have a continuous concentration of 20,000-30,000 cells/ml.

6.2.7 Removing Spat from Cultch

For some nursery systems, e.g. upwellers/downwellers, it may be desirable to remove the spat from the cultch. Several methods have been tried to remove Japanese scallop spat from cultch (Table 14) (Hodgson and Bourne, in prep.). A 500 ppm solution of sodium hypochlorite was effective in causing spat to release from the cultch within 5 minutes, a 250 ppm solution was effective after 30 minutes. A 10% solution of sodium chloride also caused spat to detach. Spat appeared healthy and alive 48 hours after treatments. Magnesium chloride and magnesium sulphate caused the spat to relax, but they remained attached to the cultch.

Another method that removed spat from cultch was to expose them to air for approximately 30 minutes, and then agitate them in a small volume of FSW. This is the method that is currently practised at the Pacific Biological Station when quantities of single spat are required.

Table 14. List of substances tested to induce detachment of spat from cultch.

Effective	
Sodium chloride	10 % in distilled H_2O
Sodium hypochlorite	550 ppm in FSW
	250 ppm in FSW
Caused spat to relax but not detach	
Magnesium chloride	10 gm/l in FSW
	66.4 gm/l in distilled H_2O
Magnesium sulfate	10 gm/l in FSW
	123.2 gm/l in distilled H_2O
Not effective	
MS 222	100 gm.l in FSW
2-Phenoxethanol	0.5 ml/l in FSW
Eponto-sombrevin	40 gm/l in FSW
Quinaldine sulfate	100 mg/l in FSW
Potassium chloride	1 gm/l in FSW
Calcium chloride	5 gm/l in FSW
Sodium lauryl sulfate	0.1 gm/l in FSW
Sodium metabisulfite	0.1 gm/l in FSW

6.3 Duration of Primary Nursery Stage

Spat should be removed from spat bags when they reach 10 mm shell height (Figure 42). Depending on the time of year, this may occur after 3-6 months for Japanese scallops and 11 months for rock scallops (Figure 43).

Handling juveniles smaller than 10 mm shell height can result in high mortalities, but they should not be left in spat bags once they have reached 10 mm. At 10 mm shell height, juvenile Japanese scallops begin to lose their ability to remain attached by byssus to the cultch and drop to the bottom of the bag. If juveniles are not removed in time, many will collect at the bottom of the bag and high mortalities will result from biting and suffocation.

When scallops are removed from spat bags, they are ready for the secondary nursery stage. Scallops can be collected by turning the spat bag inside out and gently hosing down the cultch and bag with seawater (Figure 44). Place the bag inside a shallow trough that is inclined so that as the spat are hosed off, they will wash down the trough and can be easily collected.

6.4 Secondary Nursery: 10 mm to 40-50 mm Shell Height

The secondary nursery stage is the final stage of the nursery system. Juveniles are removed from spat bags at about 10 mm shell height to prevent high mortalities, but they are too small to be placed into the growout phase. Juveniles from spat bags are placed in pearl nets or cages that have a mesh small enough to retain them. No more than 25% of the bottom surface area of the net should be occupied by scallops to allow for continued growth without causing crowding. They are suspended from a long-line and held at depths of 5 m or deeper. Nets must be checked regularly to ensure fouling does not affect growth and survival of scallops. If necessary, juvenile scallops should be resorted into additional pearl nets after 3 months to reduce density in the nets. Japanese scallops grow from 10 mm shell height to 40-50 mm shell height in 5-10 months, whereas rock scallops require up to 2 years to reach the same size.

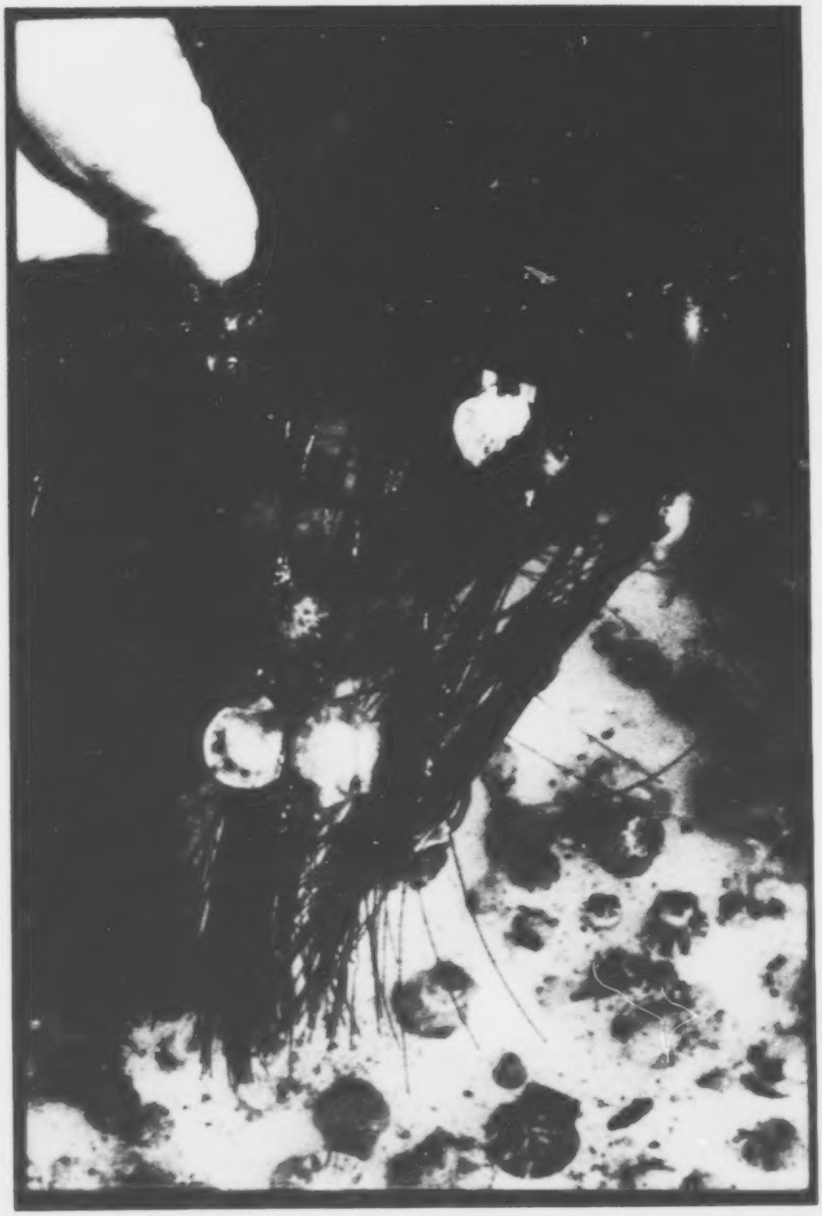

Figure 42. Juvenile Japanese scallops, 10 mm shell height.

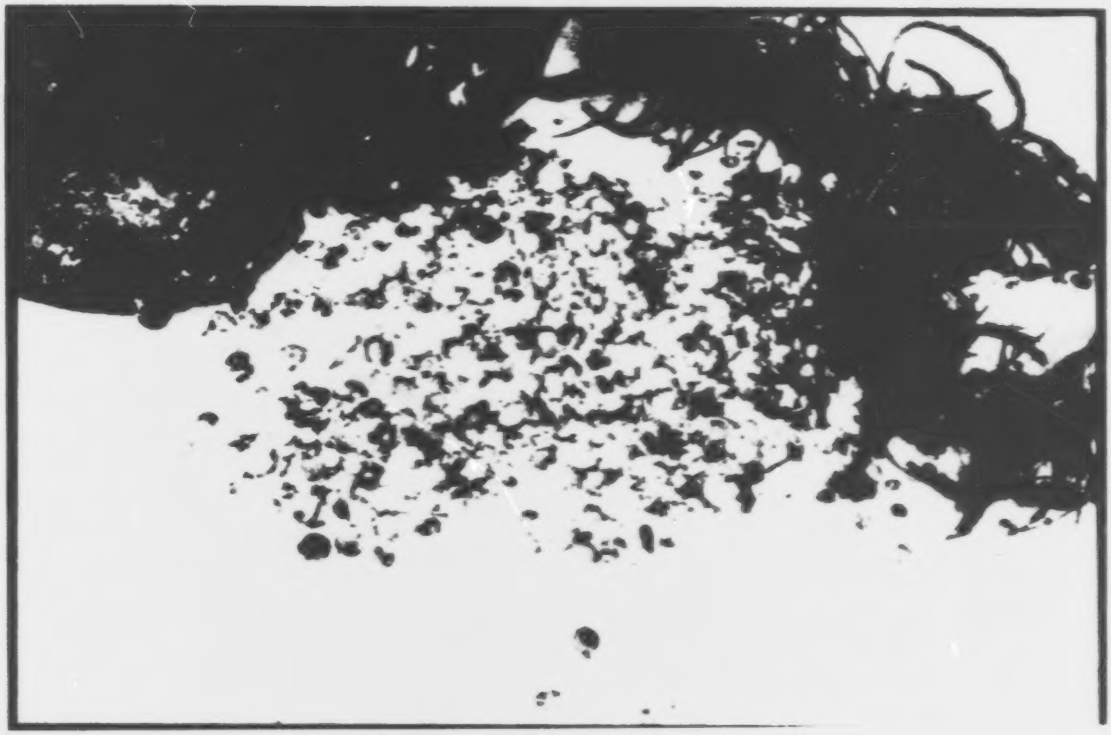

Figure 43. Juvenile Japanese scallops emptied from spat bag.

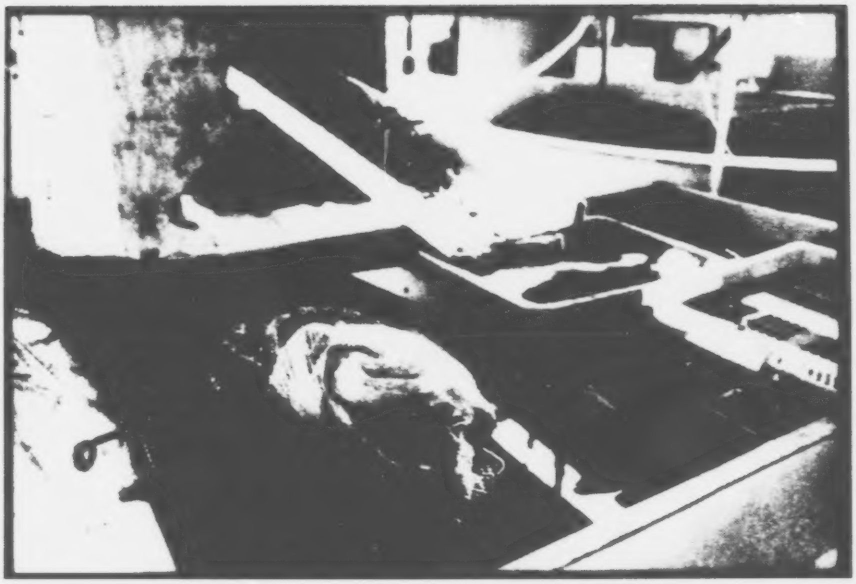

Figure 44. Apparatus for collecting and sorting juvenile scallops from spat bags. Spat are washed from cultch and into a seawater table for sorting.

7. GROWOUT

The final stage of scallop culture is the growout phase, where juvenile scallops are cultured from 40-50 mm shell height to commercial size (10 cm shell height). The growout phase ideally should be 1 year in duration. Only limited work has been undertaken in the growout phase of culture of Japanese and rock scallops at the Pacific Biological Station. Scallops can be grown by two methods, Bottom culture and hanging or suspended culture.

7.1 Bottom Culture

Bottom culture is practised in Japan and New Zealand, and is being attempted in France (Ventilla 1982; Bull 1986; Dao, et al. 1988). In Japan, juvenile scallops, 40-50 mm shell height, are released and allowed to settle on large areas of the ocean bottom to achieve densities of 5-10 scallops per square meter. They are harvested three to four years later by dragging. In New Zealand, juvenile scallops are released when they are 10-30 mm shell height and harvested 2 years later. Size of the scallop at the time of release is important to ensure they will not suffocate in the bottom substrate. Bottom type should be firm to avoid scallops sinking into the sediment.

Bottom culture has not been examined in British Columbia. It is doubtful if it has immediate potential because of the large number of benthic predators, e.g. seastars and crabs. Another complication is that leases would have to be obtained for large subtidal areas to ensure a grower had sole rights to scallops planted in an area.

7.2 Suspended Culture

Suspended or hanging culture includes methods where scallops are held in cages or nets suspended from long-lines in the water column. The long-lines should be submerged to avoid wave action (Figure 45). Long-lines must be anchored effectively. An area can be made more efficient and anchoring costs reduced if several long-lines are attached together (Figure 46).

As the weight of a long-line system increases because of growth of scallops and fouling organisms, more floatation is required at regular intervals to prevent the culture system sinking. Rather than adding more floatation, a sinker system can be used that releases weights as the culture system get heavier. Sinker weights are suspended near the bottom at regular intervals on the ground line. As the culture system sinks, one or more of the sinkers touch bottom and effectively removes the weight from the system (Figure 45).

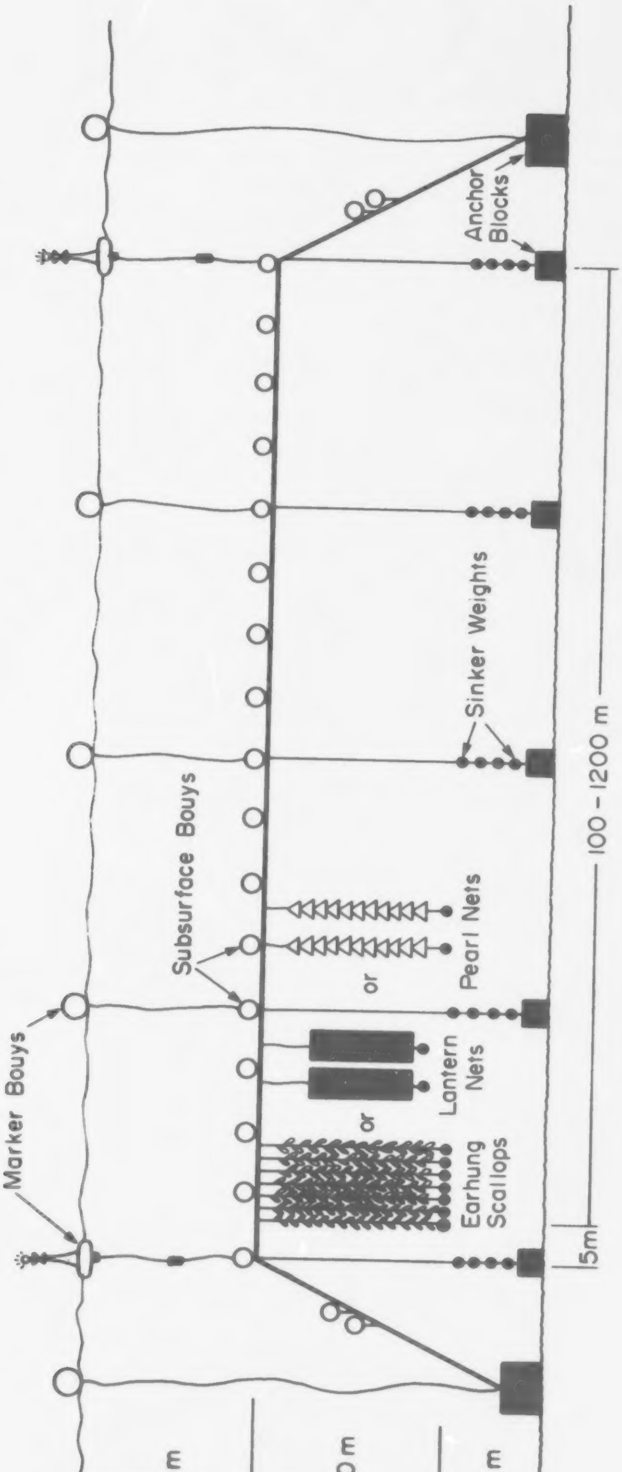
Marker Bouys
Subsurface Bouys
Anchor Blocks
Sinker Weights
Pearl Nets
or
Lantern Nets
or
Earhung Scallops
100 - 1200 m
5m
3-8 m
10-20 m
2-10 m

Figure 45. Diagram of a submerged longline system used for growout of juvenile scallops to commercial size.

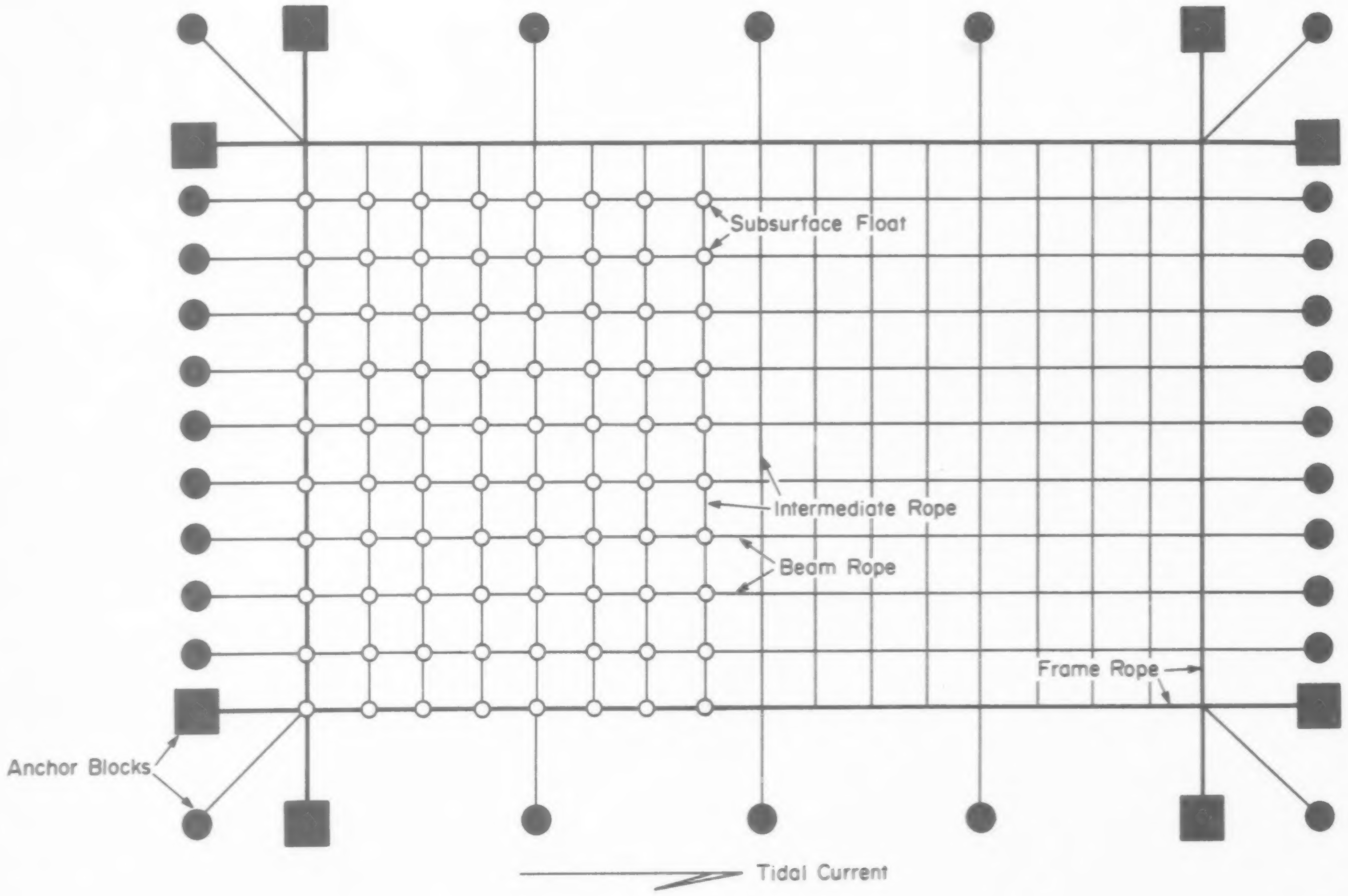
Subsurface Float
Intermediate Rope
Beam Rope
Frame Rope
Anchor Blocks
Tidal Current

Figure 46. Diagram of a multiple longline system.

7.2.1 Culture Nets

Scallops must be contained to prevent losses by swimming. The supporting surfaces of culture containers should be kept horizontal to prevent the scallops bunching into a corner. If this occurs, scallops will bite one another and this will lead to excessive mortalities. A variety of different nets and containers can be used for scallop culture. Two common types of nets are pearl and lantern nets (Figure 47).

Wire mesh enclosures are not suitable for scallop culture because the fragile shell margins can be easily broken if the scallops strike the wire mesh (Mottet 1979).

7.2.2 Stocking Density and Net Maintenance

Scallop shells, particularly juveniles, have sharp edges. If densities inside a net are too high, they will bite one another, causing damage to the soft parts. This leads to slow growth or mortalities. Stocking density will vary with site location. A rule of thumb for stocking density is that the area occupied by scallops should not be more than 25% of the bottom area of the container. A regular schedule for cleaning or changing nets and reducing scallop density should be maintained. This will be site dependent since scallop growth and fouling will vary with different sites. Initially, nets should be checked every two or three months.

7.2.3 Ear-hanging

Scallops that are 40-50 mm shell height can be ear-hung by drilling a hole in the left anterior auricle (Figure 48) and inserting a fastener that is affixed to a rope. A recent development in ear-hanging technology is the production of a small plastic clip called a-ge-pin (Figure 49) to replace the time-consuming method of threading and tying monofilament lines through scallops and attaching them to ropes.

Although ear-hanging is labour-intensive initially, it is less so overall because scallops need not be checked regularly for fouling as with net cultures. Cost of ear-hanging is about one-tenth that of net culture and up to 4.5 times as many scallops can be cultured per long-line (Mottet 1979). Valves of ear-hung scallops are more convex and contain up to 8-10% more meat than bottom-cultured animals (Mottet 1979).

7.2.4 Advantages of Suspended Culture

Experience in Japan has shown that suspended culture has advantages when compared to bottom culture (Mottet 1979). Scallops grow faster and reach market size within 2 years compared to 3-4 years for bottom culture. Suspended culture can be practised in areas not suitable for bottom culture and the scallops may bring a higher price because they contain less sand or bottom sediments.

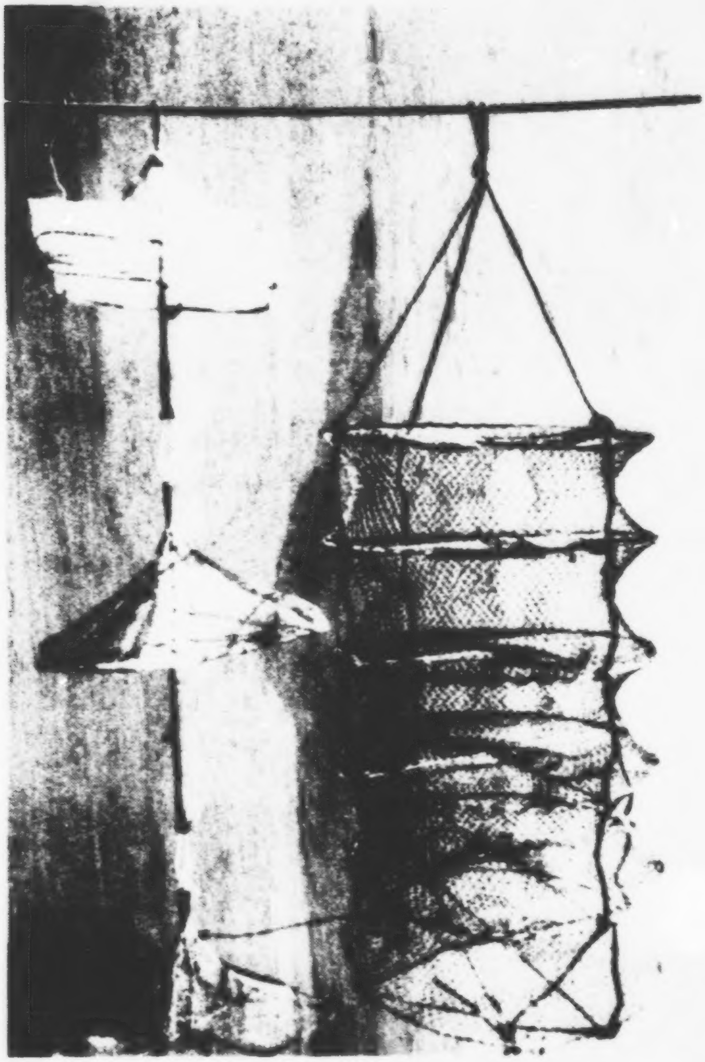

Figure 47. Pearl and lantern nets for suspended culture of scallops.

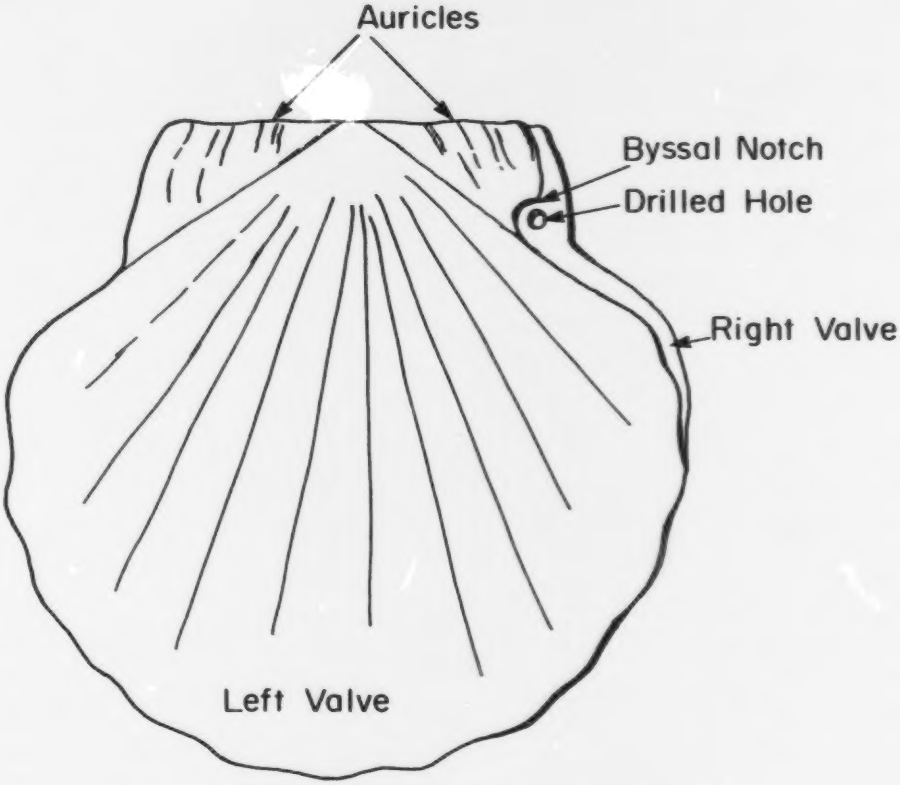
Auricles
Byssal Notch
Drilled Hole
Right Valve
Left Valve

Figure 48. Diagram of location on right valve of Japanese scallop where hole should be drilled for ear hanging culture.

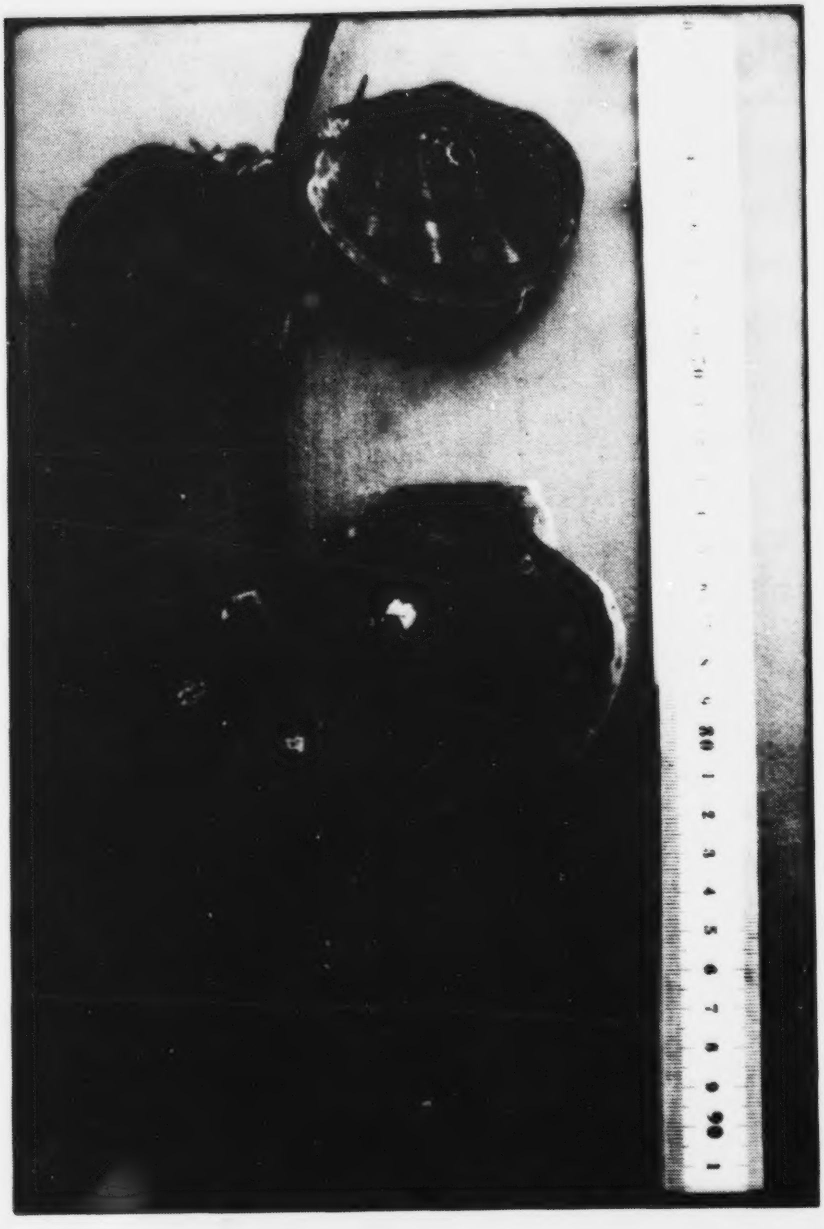

Figure 49. Scallops attached to rope by a-ge-pin for ear hanging culture.

7.3 Transportation of Scallop Seed

Frequently, seed must be shipped to a growout site. A suitable method to ship juvenile scallops is in an insulated cooler (Figure 50). They are kept moist by placing them between horizontal layers of paper towelling or foam rubber that has been wetted in cool seawater. The juveniles should be spaced so they do not touch each other and cause damage by biting. Ice packs are placed in the container to maintain a cool temperature, below 10°C. Experiments undertaken at the Pacific Biological Station show that juveniles can survive for up to 24 hours if the temperature in the cooler does not exceed 8°C (Table 15).

7.4 Site Selection

As with all culture operations, a lease must be obtained before any activity can be started. Leases are obtained from the Lands Branch of the Provincial Ministry of Lands, Parks, and Housing (Quayle 1988a).

Site selection is important and a site should be examined over a 12 month period before commercial operations are initiated. Ideally, several sites should be tested to determine which is the most suitable. Factors to be considered are both biological and non-biological and include temperature, salinity, wave action, food abundance, and disease (Table 16).

Results of preliminary growout trials with Japanese scallops indicate the following parameters are important if good growth and maximum survival are to be achieved.

7.4.1 Water Depth

Sufficient water depth is required to prevent culture lines touching the bottom at low tides which would permit predators, such as crabs and seastars, to move up the lines and prey on the scallops. Optimum water depth for hanging culture is site dependent. A minimum depth at most sites is 5 meters to avoid high surface water temperatures and low surface salinities. In Departure Bay, Japanese scallops are held at 10 m from the end of March until October to avoid warm water conditions and excessive fouling during this period, and raised to 5 m for the remainder of the year.

7.4.2 Water Temperature, Salinity and Current

Japanese scallops should be held in areas where temperatures are below 15°C and salinities are 28 ppt or greater. Higher temperatures or lower salinities can be stressful.

Rock scallops can be cultured in waters with temperatures up to 20°C and salinities of 25 ppt or greater.

Table 15. Temperature and exposure tolerances of juvenile Japanese scallops (2 cm shell height) that may be experienced during shipping.

A. Percent survival of scallops packed between layers of newsprint soaked in FSW. (2 replicates)

Temperature (°C)	Exposure (hours)		
	24	48	72
4	95	92	32
8	96	10	0
15	30	0	0

B. Percent survival of scallops immersed in FSW of different temperatures. (3 replicates)

Time (minutes)	Temperature (°C)				
	10	13	16	19	22
30	94	98	94	96	94
60	96	98	93	94	98
90	98	94	96	98	96
120	100	96	96	94	96

C. Percent survival of scallops held at 3 salinities and 3 temperatures for 120 minutes. (2 replicates)

Temperature (°C)	Salinity (ppt)		
	14	18	22
8	96	100	95
15	97	99	97
18	99	97	100

D. Percent survival of scallops emmersed from seawater. (3 replicates)

Air Temperature (°C)	Time (minutes)			
	30	60	90	120
5	98	95	99	99
15	100	100	98	100
25	98	97	96	95

Table 16. Factors that should be considered when selecting a site for growout of scallops.

Water Depth
Water Temperature and Salinity
Currents
Water Quality
Wind/Wave Exposure
Food Supply
Fouling
Disease
Bottom Type
Accessibility and Security

Figure 5C. Japanese scallops packed into styrofoam container for shipment.

A current is required to allow adequate water exchange through the culture system. Water current bring phytoplankton and dissolved nutrients to the scallops, which permit growth and survival, and help to remove faeces and detritus. Areas with no or little water current may not be suitable for scallop culture. Areas with high water current should also be avoided since they create excessive movement of the culture system. Current should range from 1.5 to 3 knots.

7.4.3 Water Quality

Water quality is important. Adequate dissolved oxygen levels must be maintained and pollution should be avoided.

Raw sewage discharge raises coliform bacteria counts in the surrounding waters but can also stimulate algal blooms because of added nutrients. These areas may be highly productive for filter-feeding scallops, but are restricted to shellfish harvesting. Scallops grown in such areas would have to be depurated before they are marketed.

Effluent from industrial sites, such as heavy metals, oil, and pulp mill effluent, can cause irreversible contamination to shellfish and can inhibit growth and survival. Sites near industrial facilities that are discharging into the seawater should be avoided.

7.4.4 Wind/Wave Exposure

Excessive wind and wave exposure can cause loss of gear and animals. A certain degree of shelter is needed at the culture site. Vertical movement of the culture system caused by wave action affects growth and survival of scallops since they are sensitive to movement and may clamp their valves closed and stop feeding (Mottet 1979). Movement of the culture system may cause animals to concentrate at one end of the culture cage, rather than maintaining a distance from each other. Vertical movement of the culture system can be reduced by selecting a sheltered growout area and/or using a submerged float line system (Section 7.2).

7.4.5 Food Supply

An adequate food supply is dependent on the productivity of the water in the general area and also the depth at which the scallops are held. Phytoplankton blooms occur in nutrient rich waters. Surface waters usually have higher concentrations of phytoplankton but it may be equally available in deeper waters in areas where vertical mixing occurs.

An simple method to test the productivity of a site is to measure growth and survival of a small number of animals and compare the results to similar trials in other areas.

7.4.6 Fouling

Fouling is caused by settlement and growth of several organisms, e.g. macroalgae, bryozoans, seasquirts, barnacles, and mussels. Heavy fouling of culture nets and scallops impedes growth of the animals, decreases the life of the gear and makes growout labour intensive (Figure 51).

Fouling can be reduced by suspending the culture system at deeper depths since most fouling usually occurs in shallow waters. Fouling is more prevalent during certain seasons and can be reduced if the culture system is managed properly. For example, in the southern Strait of Georgia, barnacle larvae set between mid-March and mid-April, usually at depths of less than 10 m (Quayle 1988a). Culture gear can be dropped to depths greater than 10 m during this time and then returned to their former depth once the set is over.

Areas with heavy fouling at all depths should be avoided if possible.

7.4.7 Disease

Limited information is available on shellfish diseases in British Columbia. To date, a protozoan parasite that may be pathogenic to scallops has been found in only one area, Booker Lagoon near Alert Bay. Mortalities caused by infestations of a polychaete worm, Polydora sp. have been recorded in the Gulf Islands area. Another disease has been recorded in scallops from the northern part of the Strait of Georgia. Also, other localized areas throughout British Columbia are known to have a prevalence of organisms pathogenic to molluscan shellfish. Before selecting a site, the shellfish disease history of the area should be investigated since massive mortalities could be a local concern.

7.4.8 Bottom

Bottom type is important when bottom culture is practised and also can affect the stability of anchorage systems for suspended culture.

Soft sediments, combined with turbulent waters, can cause turbid water conditions. Mortalities of scallops in bottom or suspended culture can result from heavy siltation (Ventilla 1982).

Moorings must be strong and well anchored in the bottom to reduce shifting of the suspended culture systems. Bottom characteristics should be investigated to ensure safe moorage.

7.4.9 Accessibility and Security

Scallop culture systems should be readily accessible year-round since they must be checked routinely.

Vandalism may be a high or low risk problem, depending on the

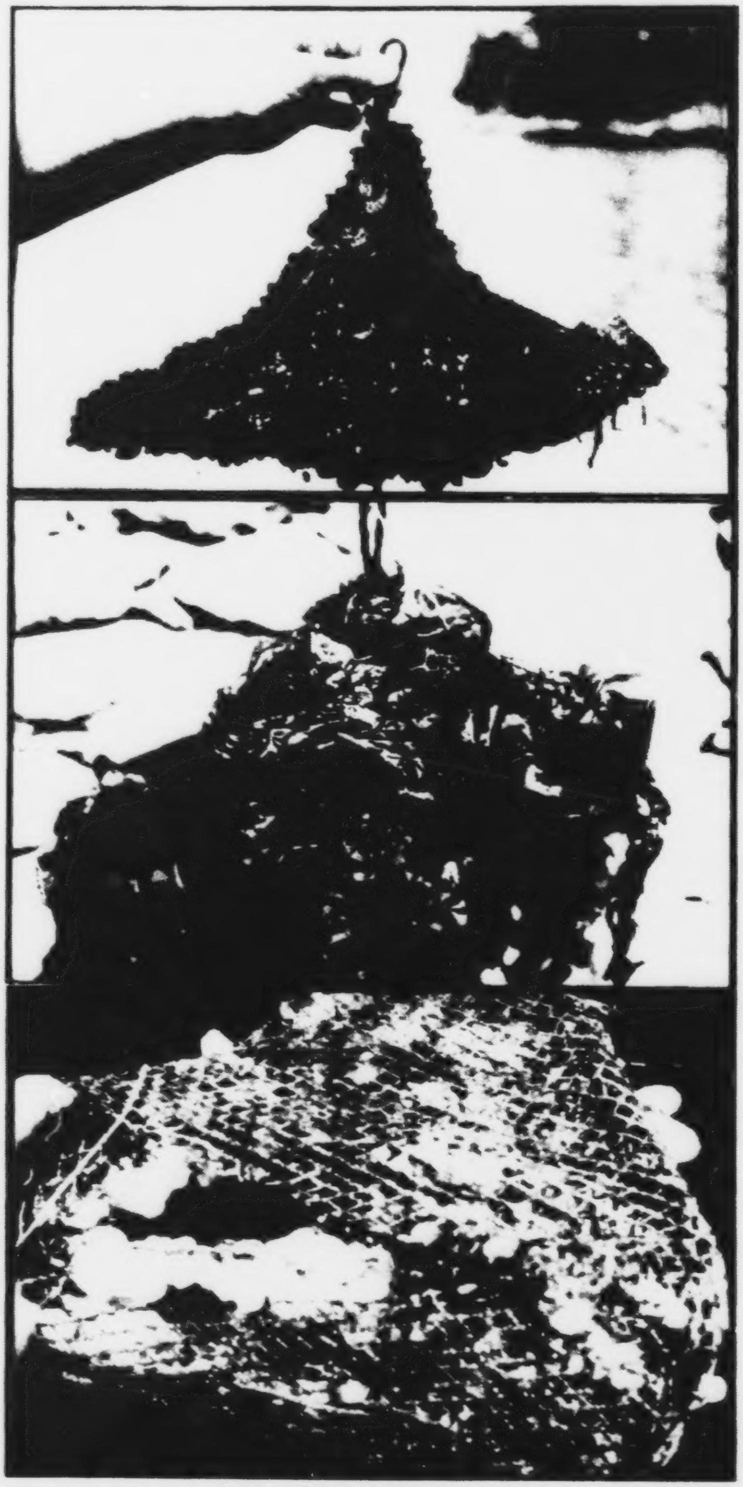

Figure 51. Three examples of heavily fouled scallop culture nets.

area. Ideally, a site should be in an area where it can be checked regularly but not with ready access to outsiders.

7.5 The British Columbia Experience

Growout studies have been undertaken with Japanese and rock scallops in British Columbia waters. Japanese scallops were cultured at 8 locations (Bourne et al. in prep). Animals were held in hanging culture nets at 3 depths, 5, 10, and 15 m suspended from floating long-lines or rafts. At 5 of the sites, scallops were reared to 10 cm shell height within 2 years (Figure 52). Mortalities ranged from 5 to 100 %, depending on the location. Some mortality is believed to have been due to excessive vertical movement since they were suspended from surface long-lines.

Growth of rock scallops was investigated in Departure Bay. Scallops were held in pearl nets, glued to plexiglass plates, or ear-hung and suspended at 5, 10, and 15 m. Preliminary analysis of the data suggest that growth to commercial size (8-10 cm shell height) occurred in three years, and survival was over 50% (Figure 52).

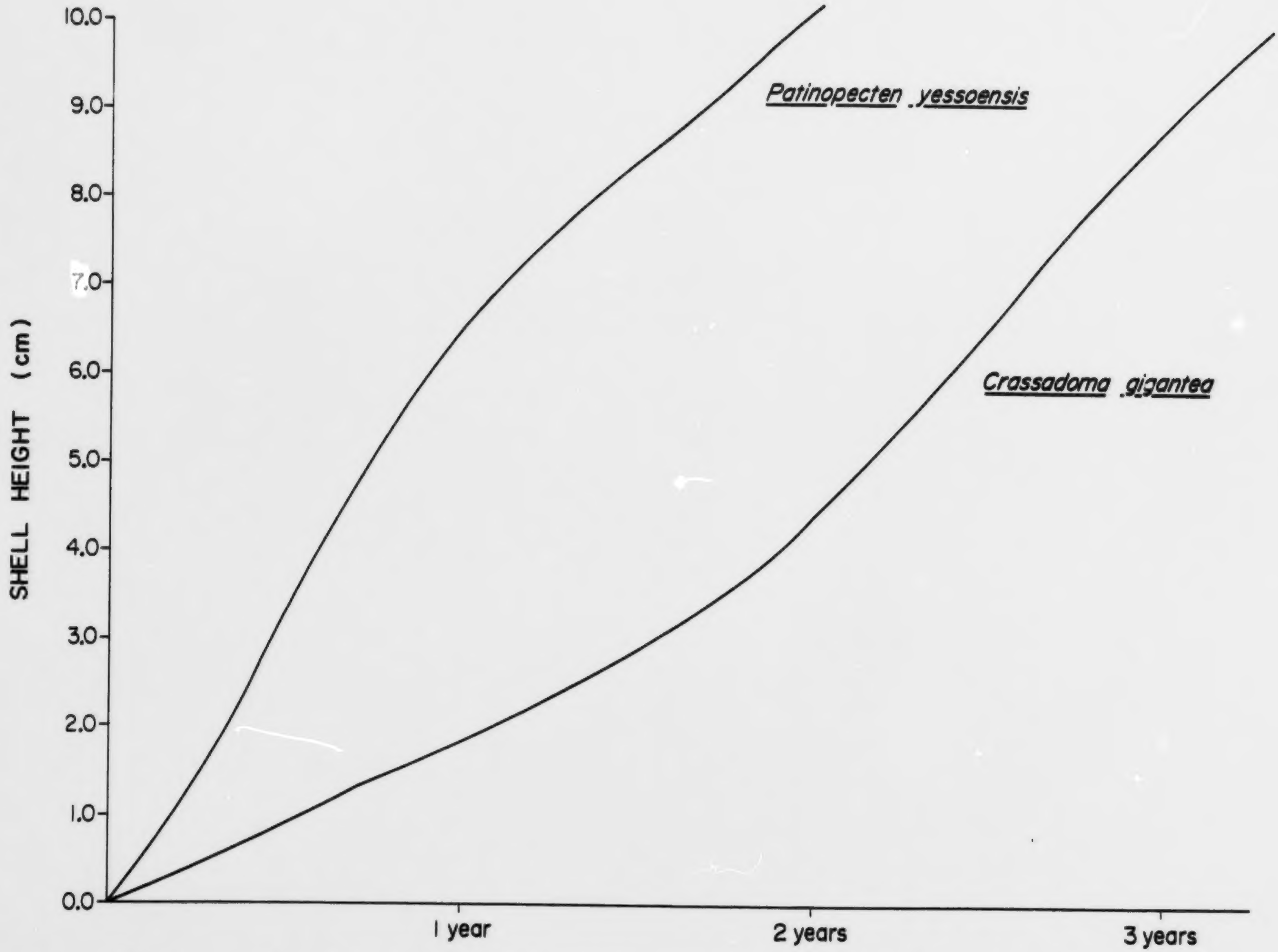

Figure 52. Average growth of Japanese, Patinopecten yessoensis, and rock, Crassadoma gigantea,

8. CONCLUSIONS

The Scallop Culture Program has shown that it is possible to culture Japanese scallops to commercial size within two years in British Columbia. Japanese scallops undergo gametogenesis in British Columbia coastal waters and hence the industry can be self-reliant for broodstock of this species.

Rock scallops can be grown to commercial size in three to four years, but this time might be decreased with better scheduling of breeding time in hatcheries.

Interest in scallop culture continues to grow in British Columbia and an industry could develop in the future. The first requisite for the industry will be an abundant and reliable supply of juveniles. One company is planning to construct a hatchery and nursery to supply the industry with juvenile scallops. Ultimate size of a scallop culture industry is unknown, but it would appear that a substantial industry could become established.

This manual has been written to assist in the development of a scallop culture industry in British Columbia. As the industry develops, further research and development will be required to make operations more efficient. New knowledge obtained from this work will form the basis for updates or revisions of this manual.

ACKNOWLEDGMENTS

Many people have been associated with or worked in the Scallop Culture Program since it began in 1981 and all have contributed to its success. We thank these people and, in particular, recognize the following people for their contribution.

We sincerely appreciate the assistance of Dr. Susumu Ito, formerly Director of the Aquaculture Centre of Aomori Prefecture, Japan, for making the necessary arrangements to import Japanese scallop broodstock. We are also indebted to Mr. Morio Takarada of the same Institution for spending six months at the Pacific Biological Station and demonstrating scallop culture technology to us. Thanks are also extended to the staff of the Canadian Embassy in Tokyo, Japan, for making the necessary arrangements to tranship scallops to Canada in Tokyo.

We give special thanks to Mr. W. Carolsfeld for his diligent work in the laboratory and for being responsible for field work, and to Mr. D. Thompson for re-organizing the culture facility to make it efficient and productive. The tireless efforts of L. Townsend, B. Kingzett and E. Downey are greatly acknowledged. Dr. S. Bower provided information regarding scallop diseases. Appreciation is extended to N. Ginter, C. Manson, K. Cooke, R. Carmichael and B. Bishop-Lowen for the time they spent with the program.

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GLOSSARY

Adductor muscle	large muscle near centre of scallop that pulls the two valves together
Algae	aquatic plants that reproduce by spores
Anterior	front or head
Auricle	with respect to scallops, the ear or wing-like projections at the hinge of a scallop (can also refer to the chamber of heart that receives blood from the body)
Axenic	culture of a single species in bacteria-free conditions
Biting	condition where shell margins of two scallops become interlocked, and subsequently damage the inner soft parts
Bivalve	mollusc of the Class Pelecypoda, having a shell of two valves that are joined by a hinge
Byssus	thread-like filament used by bivalves to attach themselves to a substrate
Cilia	a hair like structure whose rhythmic beat produces water current in bivalves
Ctendia	a leaf-like appendage that functions in respiration and filtration of food from water (used interchangeably with the term gills)
Cultch	material used to collect bivalve spat
Demibranch	single plate or leaf of a bivalve gill
Detritus	fragmented or decomposing organic material from plant and animal remains
Diatom	a single celled algae of the Class Bacillariophyceae; cells are enclosed in a siliceous shell called a frustule, cells can form chains
Dioecious	organisms in which male and females reproductive organs occur in different individuals
Diploid	the normal number of chromosomes (2n) in cells
Dorsal	the back or part of an organism away from the ground

Embryo	organism in early stages of development; in bivalves, prior to larval stage
Exhalant	area of bivalve where water currents have an outward direction
Exotic	introduced from foreign country or geographic area
Eyespot	simple organ that develops near centre of mature larvae of some bivalves and is sensitive to light
Fertilization	union of egg and sperm
FSW	filtered sea water
Flagellate	group of single-celled algae characterized by having a locomotory organ called a flagellum
Frustule	siliceous shell-like covering of a diatom
Gamete	mature, haploid, functional sex cell capable of uniting with the alternate sex cell to form a zygote
Gametogenesis	process by which eggs and sperm are produced
Gill	a leaf-like appendage that functions in respiration and filtration of food from water (see ctendia)
Halocline	the area of sharp vertical salinity change
Hinge	dorsal area of bivalve shell where two valves are joined together
Indigenous	native, not imported
Inhalant	area of bivalve where water current have an inward direction
Larva	a stage of bivalves from the embryo to metamorphosis
Ligament	fibrous spring-like material joining two valves of a bivalve at the hinge
Mantle	the soft fold enclosing the body of a bivalve which secretes the shell
Mean	average
Meiotic Division	process in which normal number of chromosomes (2n) is reduced to the haploid (n) number
Metamorphosis	in bivalves, the period of transformation from the larval to the juvenile stage

Micrometer (um)	one millionth of a meter or one thousandth of a mm
Natural Set	in bivalves, obtaining spat from spawning of natural populations
Pallial Line	faint circular line on inner surface of shell of bivalves showing location of attachment of mantle to shell
Palp	a sensory appendage near the mouth used to assist in moving food into the mouth
Pedal	pertaining to the foot
pH	a measure of acidity
Plankton	floating or weakly swimming aquatic organisms, can be phytoplankton (plants) or zooplankton (animals)
Planktotrophic	organisms that feed on phytoplankton
Polar Body	minute cells released during meiotic division of the egg after the sperm has penetrated the egg; contains excess chromosomal material to produce a haploid egg
Polyploid	animals having more than the usual number of diploid (2n) chromosomes
Posterior	the rear, away from the head
Pronuclei	in the egg, the haploid nucleus after completion of meiosis but before infusion with the sperm nucleus
Pseudofaeces	false faeces, waste material not taken into the digestive tract
Resilium	internal portion of the ligament located centrally along the hinge of a bivalve; causes the valves to open when the adductor relaxes
Salinity	the salt content of sea water usually measured in parts per thousand (ppt)
Seed	a young scallop with no specific definition to size
Settlement	behaviourial process when mature bivalve larvae seek a suitable substrate for attachment
Shell Height	in scallops, the straight line distance measured perpendicularly from the umbo to the ventral margin of the shell

Shell Length	in scallops, the straight line distance from the anterior to the posterior margins of the shell
Spat	a newly settled or attached scallop (also termed post-larval scallop)
Straight-hinge Larva	early part of larval stage, sometimes termed D-stage
Tentacle	long, unsegmented threadlike protuberance from edge of mantle that has specialized sensory function
Thermocline	the area of sharp vertical temperature change
Trochophore	planktonic stage of bivalve embryo
Umbo	beak-like projections at the dorsal part of the shell; it is the oldest part of a bivalve shell
Urogenital System	system with organs concerned with excretion (kidney) and reproduction (gonad)
Valve	one of the two parts of a bivalve shell, two valves make up one shell
Veliger Larva	the larval stage of most molluscs, characterized by the presence of a velum
Velum	ciliated locomotory organ of the molluscan veliger larva
Ventral	pertaining to the under or lower side of an animal
Zygote	diploid cell resulting from union of male and female gametes

Appendix 1. List of suppliers.

Much of the materials and equipment required for the operation of a scallop htchery can be obtained through local hardware or utility supply outlets. Other more technical or specialized equipment can be obtained from scientific supply houses, such as Western Scientific, Cole-Parmer, and Canlab.

Western Scientific Services Ltd.
11620 Horseshoe Way
Richmond B.C. V7A 4V5
(604) 274-4111

Fisher Scientific Company
196 W Third Avenue
Vancouver, B.C. V5Y 1E9
(604) 872-7641

Cole-Parmer
7425 North Park Avenue
Chicago Illinois 60648
U.S.A.
1-800-323-4340

Sigma Chemical Company
P.O. Box 14508
St. Louis, Missouri 63178
U.S.A.
1-800-325-3010

BDH Incorporated
60 East 4th Avenue
Vancouver, B.C. V5T 1E8
1-800-663-3404

Canlab
7080 River Road #131
Richmond, B.C. V6X 1X5
1-800-663-1891

However, there are a few items that can be obtained from only a few suppliers. These are listed below.

Coulter Electronics of Canada Ltd.
#6-8145-130 Street
Surrey, B.C. V3W 7X4
(604) 590-1511

Appendix 1. (cont'd.).

Supplier for coulter counter. Sometimes, a used model can be obtained.

Peacock Incorporated
2325 Burrard Street
Vancouver, B.C. V9J 3J2
(604) 731-3185

Supplier for bag filters, cartridge filters, and filter housings.

B and SH Thompson
8148 Devonshire Road
Mount Royal, Quebec H4P 2K3

Supplier for nitex screen.

Appendix 2. Calibrating an ocular micrometer

An ocular micrometer is a glass disc with a small grid or series of divisions designed to fit into an eyepiece of a microscope. When viewing through the eyepiece, the grid or scale is visible in the same plan of focus as the specimens being observed under the microscope. The scale can be used to measure the size of a specimen but first the scale must be measured itself.

A stage micrometer can be used to calibrate the ocular micrometer. The slide usually has a small measure, about 2 mm that is divided into smaller units. To calibrate the ocular micrometer, view the micrometer slide and determine the distance between each division on the ocular micrometer.

If a stage micrometer is unavailable, a rough estimate can be obtained using a small clear plastic 15 cm ruler.

Appendix 3. Operation of the saltwater pasteurizer installed at the Pacific Biological Station.

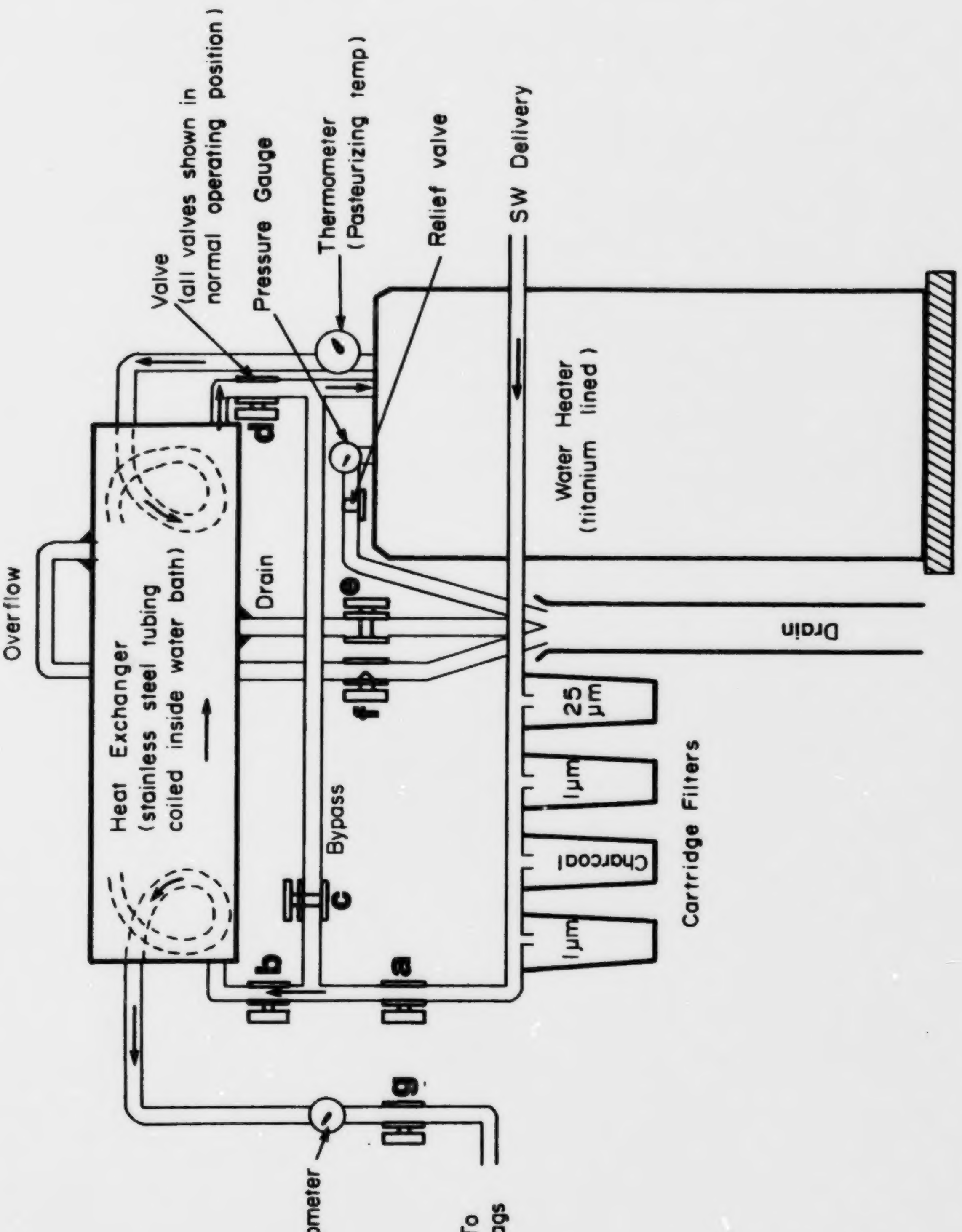
All saltwater entering the pasteurizer must be charcoal-filtered and filtered to 1 um. When not in use, a slow flow of FSW (2-3 l/min) is passed through the bypass line, through the water heater, and through the coiled line inside the heat exchanger to avoid stagnation and anoxia. The outer jacket of the heat exchanger is drained dry. When not in operation, valves A, C, E, F, and G are open and valves B and D are closed.

To operate the pasteurizer, turn on the power supply to the water heater. Turn off water flow into the heater by closing valve A. Leave for 20-30 minutes to allow the water to heat to 75-80°C. Resume slow flow of water through the heater by opening valve A.

All lines leading from the water heater must be sterilized before use by passing hot FSW (greater than 70°C) through them for at least 5-10 minutes. Valves A, C, E, F, and G are open and valves B and D are closed during this procedure.

Once all lines have been sterilized, fill the outer jacket of the heat exchanger with FSW by closing valve C and E and opening valve B. Water will pass through overflow valve F when the jacket is full. Open valve D to allow FSW to pass through the outer jacket of the heat exchanger and then into the water heater. Flow rate through the pasteurizer should be regulated to ensure outflow temperature is about 20°C. Flow can be regulated by slightly closing valve F and valve G.

After use, turn off power supply to the water heater, reduce water flow to 2-3 l/min, drain heat exchanger, open valves A, C, E, F, and G and close valves B and D.


Valve
(all valves shown in
normal operating position)
Pressure Gauge
Thermometer
(Pasteurizing temp)
Relief valve
SW Delivery
Water Heater
(titanium lined)
Overflow
Heat Exchanger
(stainless steel tubing
coiled inside water bath)
Drain
Drain
Bypass
a
b
c
d
e
f
g
25 µm
1µm
Charcoal
1µm
Cartridge Filters
Thermometer
To
Bags